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PERSPECTIVE

Rationale of renewed efforts in developing MC4R modulators to treat metabolic disorders

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Abstract The melanocortin-4 receptor (MC4R) is a key regulator of energy balance and a potential target for weight management. Early drug discovery endeavors were hindered by incomplete understanding of its signal transduction mechanisms and broad activation of G_s pathways. Recent advances indicate that MC4R elicits multiple intracellular responses, with $G_{q/11}$ signaling in the paraventricular nucleus (PVN) neurons playing a central role in appetite suppression. High-resolution cryo-electron microscopy structures of MC4R, such as the setmelanotide−MC4R−G_q complex reported here, provide valuable insights into ligand binding, receptor activation, and biased signaling, thereby enabling the design of more selective agonists with improved safety profiles. Combination therapies targeting MC4R alongside glucagon-like peptide-1 mimetics and other agents regulating metabolic pathways have shown promise to enhance weight loss. MC4R modulators are also implicated in treating other disorders, including melanocortin signaling dysfunction. These progresses call for renewed efforts in developing the next-generation MC4R-based therapies against metabolic diseases.

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1. Introduction

Obesity pharmacotherapy has entered a transformative era with the advent of glucagon-like peptide-1 (GLP-1) receptor agonists (GLP-1RAs), including liraglutide and semaglutide, and more recently dual and triple incretin-based agents such as tirzepatide and retatrutide[1-4]. These drugs produce substantial and durable weight loss by enhancing satiety, delaying gastric emptying, and improving glycemic control[5]. Despite their unprecedented efficacy, limitations remain, including gastrointestinal intolerance, interindividual variability, weight-loss plateaus, reductions in lean mass accompanying rapid weight loss, and significant weight regain after treatment discontinuation[6-8]. These residual challenges have catalyzed efforts to identify complementary therapeutic targets and to develop rational combination strategies capable of enhancing durability and metabolic precision. In this context, renewed attention has focused on central regulators of energy balance, particularly the melanocortin-4 receptor (MC4R), a class A rhodopsin-like G protein-coupled receptor (GPCR), which occupies a central position in the neuroendocrine control of energy balance, nutrient sensing, and feeding behavior[9-13]. Since its cloning in 1993, MC4R has been established as a key molecular node through which hypothalamic circuits integrate hormonal, nutritional, and neuronal signals to coordinate food intake, thermogenesis, glucose metabolism, sympathetic outflow, cardiovascular function, and linear growth[14-18]. Among the five melanocortin receptor subtypes, MC4R is intimately linked to body weight regulation, with loss-of-function (LoF) mutations representing the most common monogenic cause of early-onset obesity in humans[19-22]. Conversely, rare gain-of-function (GoF) variants confer resistance to obesity and improved metabolic profiles[23,24]. These clinical observations highlight MC4R not only as a fundamental biological regulator of energy homeostasis but also as a highly attractive therapeutic target.

Physiologically, MC4R function is embedded within the melanocortin system: a hierarchical, evolutionarily conserved network defined by the antagonistic actions of proopiomelanocortin (POMC)-derived melanocyte-stimulating hormones (MSHs), which act as endogenous agonists, and agouti-related peptide (AgRP), which functions both as an antagonist and an inverse agonist (Fig. 1)[25-27]. The anatomical architecture of this system is dominated by two interoceptive neuronal populations within the arcuate nucleus of the hypothalamus (ARC): POMC neurons that suppress feeding and promote negative energy balance, and AgRP/NPY/GABA neurons that stimulate feeding and conserve energy[28]. These first-order neurons project to second-order MC4R-expressing neurons located in key hypothalamic and extra-hypothalamic nuclei, including the paraventricular nucleus (PVN), lateral hypothalamus, bed nucleus of the stria terminalis, and brainstem regulatory centers[29]. Through these distributed circuits, MC4R works as an integrative switch that translates peripheral metabolic cues, such as leptin, insulin, ghrelin, GLP-1, and gastrointestinal satiety signals into coherent behavioral and autonomic responses[30,31].

For more than two decades, MC4R signaling was predominantly understood through the classical GPCR "lock-and-key" paradigm, in which activation by endogenous ligands was thought to primarily couple the receptor to G_s proteins, stimulate cyclic 3′,5′-adenosine monophosphate (cAMP) production, and activate protein kinase A (PKA)[32]. The severe cardiovascular side-effects observed in preclinical and clinical studies of early MC4R agonists, including melanotan II (MTII), tetrahydroisoquinoline (THIQ), and other peptide and small-molecule compounds, underscored the limitations of this one-pathway interpretation[22,33-37]. Subsequent advances in molecular pharmacology, structural biology, and single-cell neurocircuit dissection have unveiled a far more nuanced signaling landscape[11,38-40]. MC4R is now recognized as a prototypical example of GPCR-biased signaling. This refers to the ability of distinct ligands to selectively stabilize receptor conformations that favor specific intracellular signaling pathways, including those mediated by G_s, $G_{q/11}$, G_i, and β-arrestins, as well as G protein-independent modulation of ion channels such as the potassium inward rectifier channel 7.1 (Kir7.1)[41,42].

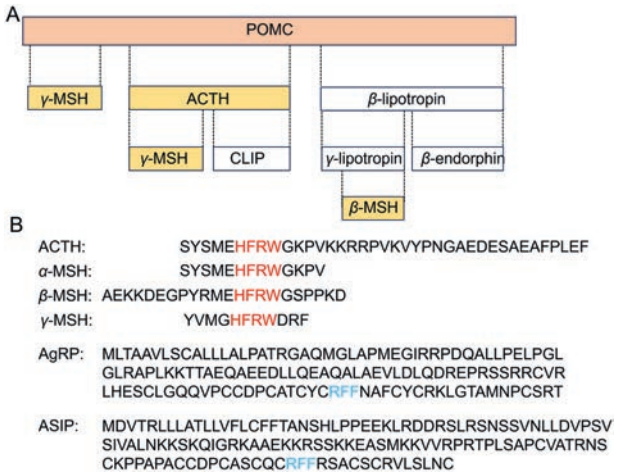

Figure 1 Endogenous ligands of MCRs. (A) Schematic representation of the processing of the precursor POMC into melanocortins. CLIP: corticotropin-like intermediate lobe peptide. (B) Amino acid sequences of endogenous ligands for MCRs.

A paradigm-shifting aspect of this complexity is the realization that food intake is preferentially regulated by a naturally occurring $G_{q/11}$-biased MC4R signaling axis, largely confined to PVN MC4R-expressing neurons. Genetic ablation of $G_{q/11}$ in PVN neurons results in severe hyperphagic obesity without impairing energy expenditure, whereas G_s deficiency affects thermogenesis and metabolic rate but leaves feeding behavior intact[43,44]. These findings, along with further demonstrations that endogenous antagonist/inverse agonist AgRP could bias MC4R signaling toward G_i pathways, reshaped the conceptual framework for developing MC4R-targeted weight loss agents[45-47].

The landscape of MC4R structural biology has undergone transformative progress in recent years due to breakthroughs in cryo-electron microscopy (cryo-EM), enabling high-resolution visualization of MC4R in complex with diverse agonists, antagonists, and G proteins (Table 1[38,48-53]). These structures reveal unique three-dimensional features, including an unusually hydrated ligand-binding pocket, distinct ionic interactions critical for receptor activation, and conformational signatures that discriminate G_s-coupled states from G_q-biased states. We have newly determined the cryo-EM structure of the setmelanotide−MC4R−G_q complex at a global resolution of 3.11 Å, which fills a major gap in this field by defining, at atomic detail, the molecular determinants that enable G_q-biased signaling and dissociate appetite suppression from cardiovascular stimulation.

The development and recent U.S. Food and Drug Administration (FDA) approval of the G_q-biased MC4R agonist setmelanotide has clinically validated the concept that selective engagement of MC4R signaling pathways can achieve potent appetite suppression with minimized adverse cardiovascular effects[54,55]. This

Table 1 Summary of the resolved MC4R structures

PDB ID	Complex	Receptor conformation	Ligand	Resolution (Å)	Method	Ca^{2+} dependence	Year	Ref.
6W25	SHU9119−MC4R	Inactive	SHU9119: Non-selective peptide antagonist	2.8	X-ray	Yes	2020	52
7PIV/7F54	Afamelanotide−MC4R−G_s	Active	Afamelanotide: Non-selective cyclic peptide agonist	2.9/3.0	Cryo-EM	Yes	2021	51/50
7PIU/7AUE	Setmelanotide−MC4R−G_s	Active	Setmelanotide: Partially G_q-biased peptide agonist	2.6/3.0	Cryo-EM	Yes	2021	51/38
7F53	α-MSH−MC4R−G_s	Active	α-MSH: Non-selective peptide agonist	3.0	Cryo-EM	Yes	2021	50
7F55	Bremelanotide−MC4R−G_s	Active	Bremelanotide: Non-selective peptide agonist	3.1	Cryo-EM	Yes	2021	50
7F58	THIQ−MC4R−G_s	Active	THIQ: Selective small-molecule agonist	3.1	Cryo-EM	Yes	2021	50
8WKY	PG-934−MC4R	Inactive	PG-934: Partially selective peptide antagonist	2.9	X-ray	Yes	2024	48
8WKZ	SBL-MC-31−MC4R	Inactive	SBL-MC-31: Selective peptide antagonist	3.3	X-ray	Yes	2024	48
8QJ2	pN162−MC4R−G_s	Active	pN162: Selective nanobody agonist	3.4	Cryo-EM	No	2024	49
9K3K	Unliganded MC4R−G_s	Intermediate	None	3.1	Cryo-EM	No	2025	53

breakthrough accelerated a new era of rational design for biased agonists, allosteric modulators, and structure-guided pharmacotherapeutics targeting MC4R for rare monogenic obesity disorders and potentially for broader metabolic indications.

In this perspective, we synthesize historical, mechanistic, structural, pharmacological, and translational perspectives on MC4R, with a specific emphasis on biased signaling as a unified principle that explains receptor pleiotropy and informs next-generation drug discovery. We begin by outlining the historical trajectory of MC4R research and the evolution of pharmacological understanding, followed by a detailed examination of canonical and noncanonical signaling cascades mediated by MC4R. We then conduct an in-depth exploration of structural breakthroughs, including that of newly achieved. Finally, we discuss clinical translation and future directions for leveraging biased signaling to discover safer and more effective therapeutics.

2. Historical path

The discovery and scientific evolution of MC4R reflect a broader trajectory of modern neuroendocrinology and GPCR pharmacology. Long before MC4R was molecularly identified, the conceptual foundations of the melanocortin pathway were shaped by early observations that pituitary extracts influenced pigmentation, feeding behavior, and metabolic processes[56]. With the identification of α-MSH as a POMC-derived peptide in the 1970s[57], it became clear that melanocortins exerted wide-ranging central effects. Yet, the molecular mediators of these actions remained unknown until the early 1990s. The cloning of the five melanocortin receptors (MC1R−MC5R) opened a new era, with MC4R cloned in 1993, rapidly emerging as the central regulator of energy balance in the brain[18,58-60].

Early functional studies established MC4R as a critical node in homeostatic feeding regulation. The receptor is highly expressed in the hypothalamic nuclei central to appetite and autonomic control, particularly PVN. Genetic analyses soon revealed that mice lacking MC4R exhibit severe hyperphagia, early-onset obesity, increased linear growth, impaired autonomic output, and altered glucose homeostasis[61]. Human genetic studies subsequently showed that heterozygous LoF MC4R mutations are the most common monogenic cause of severe childhood obesity, firmly positioning MC4R as an essential effector of melanocortinergic tone and a high-value therapeutic target[62,63].

These discoveries catalyzed the first major wave of MC4R drug development beginning in the late 1990s. Pharmaceutical interest surged as MC4R agonists such as MTII demonstrated potent anorectic effects in rodent models[64,65]. Drug discovery efforts focused on peptide derivatives, cyclic analogues, and small-molecule agonists, including THIQ, LY2112688, MC4-NN-0453, bremelanotide derivatives, and other early entities[33,66]. The prevailing pharmacological paradigm held that MC4R signaling operated almost exclusively through the G_s−cAMP−PKA axis. Thus, ligand optimization centered on cAMP readouts, with the assumption that maximal G_s stimulation would yield optimal appetite suppression.

However, the clinical development of these early compounds uncovered major limitations. Many agonists caused marked cardiovascular side-effects, nausea, and sexual arousal, which overshadowed their anorectic potential[35]. In many cases, these adverse events were observed even at doses insufficient for meaningful weight loss. Meanwhile, an emerging body of evidence showed

that some human obesity-associated MC4R variants retained normal cAMP signaling, challenging the assumption that G_s activation alone governed metabolic outcomes. This divergence between molecular pharmacology and clinical phenotype highlights a fundamental knowledge gap: the MC4R signaling landscape is far more complex than the original "one receptor—one pathway" model implied.

The setbacks led to prolonged stagnation in MC4R research throughout the 2000s and early 2010s. Without structural templates for rational design and without tools to interrogate biased signaling, many MC4R programs were deprioritized or terminated[67]. The field entered a state of uncertainty, as drug developers questioned whether it was possible to dissociate MC4R-mediated appetite suppression from undesirable autonomic and cardiovascular outcomes.

The renaissance of MC4R research in the late 2010s was driven by a confluence of conceptual, structural, and translational advances. A major turning point was the recognition that MC4R engages multiple intracellular pathways, including G_s, $G_{q/11}$, $G_{i/o}$, β-arrestins, and ion-channel-coupled mechanisms[68,69]. Crucially, genetic studies showed that appetite suppression is mediated predominantly through $G_{q/11}$ signaling within PVN MC4R neurons, whereas G_s signaling primarily drives energy expenditure and cardiovascular responses. This clarified why early G_s-biased agonists produced significant side-effects while failing to achieve adequate weight reduction. The idea that biased agonism, not simply receptor selectivity, determines therapeutic success fundamentally reframed MC4R drug discovery.

In the meantime, the global obesity therapeutic landscape underwent a profound transformation due to the unprecedented success of GLP-1RAs, including liraglutide, semaglutide, and later dual and triple incretin mimetics such as tirzepatide and retatrutide[1,3,70,71]. These agents demonstrated remarkable clinical efficacy and quickly expanded the anti-obesity market to an unprecedented scale. This rapid growth created renewed demand for complementary mechanisms capable of enhancing or extending the weight-loss effects of incretin-based therapies. MC4R emerged as one of the most compelling targets because melanocortinergic appetite suppression acts at a downstream and mechanistically distinct node from GLP-1R signaling[72-74]. GLP-1RAs influence satiety, gastric motility, glycemic control, and reward circuitry, whereas MC4R directly controls homeostatic feeding circuits and energy allocation[75]. Importantly, both preclinical and emerging clinical evidence demonstrate that co-activation of GLP-1R and MC4R produces additive, even synergistic, weight-loss effects[74]. These findings position MC4R as a rational partner pathway for next-generation combination therapies designed to sustain or deepen weight reduction, especially in patients who have reached plateau efficacy following GLP-1RA treatment.

In parallel, transformative cryo-EM studies enabled the determination of high-resolution MC4R structures bound to endogenous peptides, synthetic agonists, antagonists, and heterotrimeric G_s proteins. These structures revealed key determinants of ligand specificity, ionic activation switches, and conformational signatures associated with G_s- *versus* G_q-biased states[38,48-52].

Finally, the FDA approval of setmelanotide validated MC4R as a therapeutically viable and pharmacologically tractable target. Its potent anorectic efficacy, strong safety profile, and intrinsic G_q-biased signaling redefined expectations in the field, directly countering the skepticism stemming from earlier drug development failures. The success of setmelanotide catalyzed renewed industrial investment into MC4R agonists, biased ligands, allosteric modulators, and combination therapeutics, favorably establishing MC4R as an essential component of a new era in weight management.

Together, the historical arc of MC4R research—from early discovery, to rapid rise and subsequent stagnation, and finally to its current resurgence—illustrates the transformative power of mechanistic insight, structural understanding, and market-driven translational need. MC4R has re-emerged as a cornerstone not only in energy balance regulation but also in designing multi-pathway therapeutic strategies to combat obesity.

3. Signaling pathway

MC4R exhibits an unusually diverse signaling repertoire coordinating multiple intracellular pathways to regulate energy balance, glucose metabolism, sympathetic function, blood pressure, and neurobehavioral states[20,36,42,76-79]. Although MC4R was originally characterized as a canonical G_s-coupled receptor, it is now evident that its physiological effects arise from a far more intricate network of downstream signals, including $G_{q/11}$-, $G_{i/o}$- and β-arrestin-mediated processes, as well as direct modulation by ion channels such as Kir7.1[68,69,80,81]. The interplay of these pathways and the capacity of individual ligands to selectively engage them provide a mechanistic explanation for the varied metabolic and cardiovascular outcomes associated with MC4R activation. Understanding this multi-dimensional signaling architecture is essential for interpreting historical findings and for guiding the development of next-generation MC4R therapeutics.

Early studies assumed that MC4R-mediated appetite suppression and metabolic improvements predominantly originated from G_s activation and cAMP accumulation[82,83]. Indeed, G_s signaling plays an important role in enhancing sympathetic output, stimulating brown adipose tissue thermogenesis, and increasing whole-body energy expenditure[10]. This canonical pathway has been rigorously validated through complementary experimental approaches. For instance, selective activation of G_s signaling in MC4R neurons using the chemogenetic G_s-coupled designer receptor exclusively activated by designer drugs (G_s-DREADD) system significantly reduced food intake and increased energy expenditure, as evidenced by elevated oxygen consumption and fatty acid oxidation[84]. These gain-of-function findings are corroborated by loss-of-function studies: MC4R-specific G_s knockout mice (MC4R−G_sKO) exhibit hyperphagia, reduced energy expenditure, impaired thermogenesis, and blunted anorectic responses to melanotan-II, collectively establishing G_s signaling as a central mediator of MC4R metabolic actions[44]. Activation of $G\alpha_s$ stimulates adenylyl cyclase (AC), thereby elevating cAMP, and PKA, which in turn regulate transcriptional activities *via* cAMP response element-binding protein (CREB) and orchestrate diverse metabolic pathways, including energy and lipid metabolism[85,86] (Fig. 2). However, this canonical pathway also underlies many of the adverse cardiovascular effects observed with MC4R agonists. *In vitro* and *in vivo* studies have shown that non-selective agonists that strongly activate G_s typically induce increases in blood pressure, heart rate, and sympathetic tone, rendering them unsuitable for long-term metabolic therapy[44,87,88]. Recent investigations have extended this understanding by revealing MC4R expression beyond classical hypothalamic circuits to peripheral autonomic sensors. In spontaneously hypertensive rats (SHR), MC4R is functionally expressed in carotid body arterial chemoreceptors, where melanocortin agonists

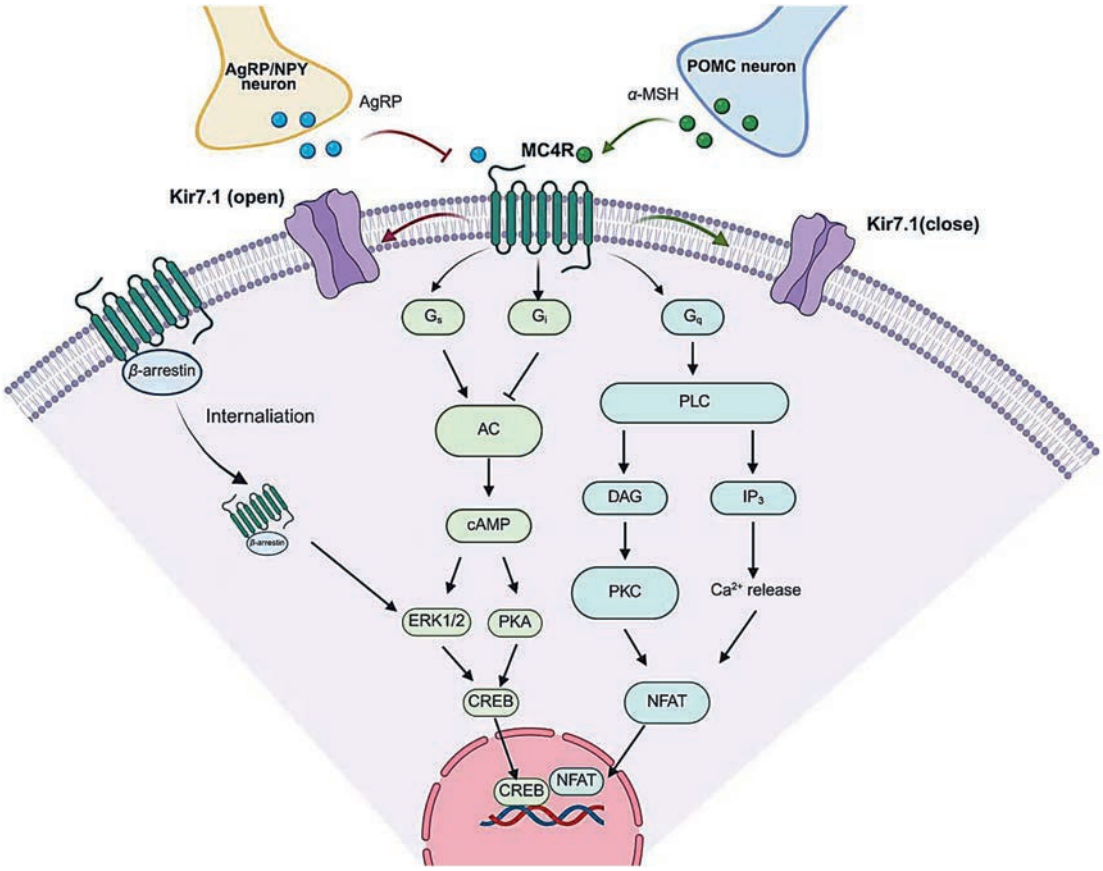

Figure 2 Most important signaling pathways mediated by MC4R. MC4R activation through the G_s pathway enhances AC activity, elevates intracellular cAMP levels, and promotes the nuclear translocation of CREB, thereby modulating downstream gene transcription. G_i provides an opposing influence by dampening AC signaling. Engagement of the G_q/PLC pathway induces intracellular Ca^{2+} release. β-arrestin facilitates the internalization of activated MC4R. The inward-rectifier potassium channel Kir7.1 closes in response to α-MSH and opens in the presence of AgRP, providing a G-protein-independent regulatory mechanism. ERK1/2, extracellular signal-regulated kinases 1 and 2; DAG, diacylglycerol; IP_3, inositol 1,4,5-trisphosphate; NFAT, nuclear factor of activated T cells; NPY, neuropeptide Y. This figure was created by https://www.biorender.com/.

activate these cells to potentiate both resting and chemoreflex-evoked sympathetic activity[89]. This finding implicates MC4R signaling in arterial chemosensation as a previously unrecognized contributor to sympathetic overactivity in cardiometabolic disease, highlighting the need for pathway-selective therapeutics that preserve metabolic benefits while minimizing cardiovascular off-target effects. Importantly, subsequent genetic studies demonstrated that the anorectic effects traditionally attributed to G_s signaling are not solely dependent on G_s itself. MC4R is highly expressed in PVN[13,39]. Mice with G_s deficiency in PVN showed no significant changes in food intake, energy expenditure, or body length, and exhibited no primary defects in glucose metabolism[90]. In contrast, PVN-specific loss of $G_{q/11}$, which stimulates phospholipase C (PLC), results in severe hyperphagic obesity[44]. These findings prompted a reassessment of which pathways are truly responsible for physiological actions of MC4R.

A pivotal shift occurred when researchers identified $G_{q/11}$ signaling as the principal mediator of appetite suppression downstream of MC4R. Conditional deletion of $G_{q/11}$ in PVN neurons produced profound hyperphagia and obesity, exhibiting the MC4R knockout phenotype[44]. This discovery revealed that MC4R-driven regulation of feeding depends critically on PLC activation, inositol trisphosphate-mediated calcium mobilization, and downstream protein kinase C (PKC) signaling[91]. Furthermore, mice carrying a human obesity-associated MC4R mutation ($F51^{1.39}L$, superscripts refer to Ballesteros−Weinstein numbering[92]) that selectively disrupts $G_{q/11}$ signaling, but preserves G_s function, displayed marked hyperphagia and obesity, confirming to a large extent that PLC−IP_3−Ca^{2+}−NFAT signaling is responsible for MC4R-regulated feeding[43]. These events shape neuronal excitability and synaptic integration in hypothalamic circuits controlling hunger and satiety. Mediators of the G_q cascade depolarize MC4R-expressing neurons and potentiate excitatory output to downstream satiety centers. In contrast to the cardiovascular liabilities associated with G_s activation, G_q engagement produces potent anorectic responses without marked sympathetic stimulation. This dissociation explains why ligands such as setmelanotide, with G_q bias and reduced G_s efficacy, achieve robust weight loss while maintaining a favorable cardiovascular safety profile[55,93,94].

In parallel with the discovery of G_q signaling, scientists uncovered the roles of G_i signaling pathways in MC4R functionality (Fig. 2). AgRP, long viewed solely as a competitive antagonist, also operates as an inverse agonist that decreases basal G_s activity and can bias the receptor toward G_i coupling[95-100]. Activation of G_i leads to inhibition of AC, suppression of cAMP production, and hyperpolarization of neurons within the melanocortin circuitry. These changes promote feeding behavior and favor anabolic metabolic states, supporting the function of AgRP as a potent

orexigenic signal during fasting. The ability of MC4R to engage G_i adds an additional layer of bidirectional regulation in the melanocortin system, further illustrating how ligand structure and conformation dictate divergent physiological outputs.

Beyond heterotrimeric G proteins, MC4R also activates G protein−independent pathways mediated by β-arrestins[101] (Fig. 2). Traditionally associated with receptor desensitization and internalization, β-arrestins are now recognized as scaffolds for alternative signaling cascades. A large-scale genomic analysis of approximately 500,000 individuals from the UK Biobank revealed that a subset of human gain-of-function MC4R variants exhibit biased signaling toward β-arrestin recruitment [23]. Notably, this β-arrestin-biased signaling profile was associated with lower BMI and reduced risk of obesity, type 2 diabetes, and coronary artery disease, suggesting that β-arrestin pathway engagement may confer cardiometabolic protection independent of canonical G protein activation. Complementary genetic studies in mice reinforce this concept: selective deletion of β-arrestin 2 in MC4R-expressing neurons (MC4R-β-arr2-KO) resulted in increased food intake, adiposity, and insulin resistance, while targeted re-expression of β-arrestin 2 in the paraventricular nucleus restored normal anorectic responses[102]. Although the precise molecular mechanisms linking β-arrestin signaling to energy balance remain incompletely characterized, emerging evidence suggests that β-arrestin may modulate MC4R subcellular trafficking, particularly its dynamic localization within primary cilia—specialized signaling organelles where receptor availability directly influences neuronal responsiveness to melanocortin peptides[103]. Nevertheless, the downstream effectors and signaling nodes through which β-arrestin exerts its metabolic effects warrant further investigation.

One of the most distinctive features of MC4R signaling is its regulation of Kir7.1[81,104] (Fig. 2). In PVN neurons, α-MSH activates MC4R to engage a G protein-independent coupling with Kir7.1, whose closure depolarizes the membrane and increases neuronal firing[81]. Conversely, AgRP enhances Kir7.1-mediated K^+ efflux, producing hyperpolarization and decreased neuronal activity. This complementary action of a key ion channel provides an elegant, G protein-independent mechanism by which endogenous melanocortin peptides exert opposing physiological effects[82,105]. The physiological relevance of this pathway is substantiated by *in vivo* genetic models: mice with targeted Kir7.1 deletion specifically in MC4R-expressing cells exhibit resistance to MC4R agonist-induced PVN neuron depolarization, blunted anorectic responses, late-onset obesity, accelerated linear growth, and glucose intolerance[80]. These phenotypes indicate that Kir7.1 contributes to a distinct subset of MC4R's physiological functions, operating in parallel with G protein cascades as a fast-acting modulatory system that enables rapid and precise control of neuronal output. Despite the established functional interaction between MC4R and Kir7.1, fundamental mechanistic questions remain unresolved. It is currently unknown whether this coupling is direct or mediated by intermediary proteins such as β-arrestins or other scaffolding molecules, and the molecular and structural basis underlying MC4R-mediated Kir7.1 modulation awaits definitive characterization[68,106-108]. Obviously, such a multidimensional framework positions MC4R as an integrative node coordinating homeostatic feeding, autonomic output, and metabolic regulation. Ligand-specific stabilization of receptor conformations determines the preferential activation of G_s, $G_{q/11}$, $G_{i/o}$, or β-arrestin-related pathways, each producing a unique physiological signature. Synthetic agonists thus differ not only in receptor affinity and potency but also in their ability to engage specific routes of these pathways. This conceptual shift from a single canonical pathway to a multifunctional signaling system has been essential for understanding the divergent outcomes of early MC4R drug discovery programs and for guiding the development of selective biased agonists capable of dissociating therapeutic effects from adverse responses. α-MSH, for example, activates both G_s and G_q moderately, producing a balanced anorectic effect with physiological sympathetic activation. Setmelanotide, by contrast, preferentially stabilizes conformations that favor G_q coupling while engaging G_s[109]. This bias causes potent satiety without cardiovascular stimulation. AgRP stabilizes inverse conformations of the receptor, suppressing G_s activity and biasing toward $G_{i/o}$[110]. Small-molecule agonists developed in earlier clinical programs often favored G_s activation, explaining their limited tolerability and failure to produce sustained patient benefits[36]. The ability to direct signaling bias through ligand structure has therefore changed the focus of MC4R pharmacology from "activation *versus* inhibition" to "which pathway is activated, and to what degree". Compared with the well-characterized G protein pathways, the molecular architecture underlying β-arrestin- and Kir7.1-mediated signaling remains relatively underexplored and supported by more limited evidence. Although genetic and electrophysiological studies in mice clearly implicate β-arrestin 2 and Kir7.1 in specific MC4R functions, the precise downstream signaling cascades, intermediary proteins, and structural determinants governing these non-canonical pathways have yet to be defined. Elucidating how these distinct signaling modalities are integrated into the broader MC4R signaling network—and how they can be selectively modulated by biased ligands—represents an important frontier for developing next-generation therapeutics with improved efficacy and safety profiles. In summary, MC4R is a paradigmatic example of a GPCR whose physiological functions arise from a rich and highly tunable signaling landscape. The receptor integrates multiple G protein pathways, ion channel modulation, and arrestin dynamics, and each signaling branch contributes uniquely to metabolic regulation. Ligand-specific conformational selection fundamentally determines which pathways dominate, and this mechanistic principle has emerged as the central framework for interpreting both historical clinical data and contemporary therapeutic development. This integrated view sets the stage for in-depth exploration of the structural basis of MC4R signaling.

4. Structural characteristics

Structural studies on MC4R progressed rapidly in the past few years, informing us of the molecular details governing ligand recognition, receptor activation, and signal transduction (Table 1). We now understand how endogenous peptides, synthetic analogs, and small molecules differentially activate the receptor displaying unique architectural features and diverse ligand-induced conformational states.

A foundational breakthrough occurred with the determination of the first MC4R crystal structure bound to antagonist SHU9119 at 2.8 Å resolution (PDB: 6W25)[52]. This milestone defined several unusual characteristics of MC4R that distinguish it from canonical class A GPCRs. Chief among these is an essential Ca^{2+} positioned within the orthosteric pocket, coordinated by residues $E100^{2.60}$, $D122^{3.25}$, and $D126^{3.29}$. Functional analyses demonstrated that extracellular Ca^{2+} dramatically enhances α-MSH affinity and potency, establishing a rare cofactor-dependent activation mechanism among GPCRs. Additional structural features include an exceptionally short extracellular loop 2 (ECL2), the absence of a conserved ECL2−transmembrane helix 3

(TM3) disulfide bridge, and an enlarged extracellular vestibule capable of accommodating bulky melanocortin peptides. Notably, the crystallized receptor construct retained the ability to modulate Kir7.1 potassium channel independent of heterotrimeric G proteins, confirming the presence of noncanonical signaling mechanisms. Interestingly, for the entire MCR family with high sequence similarity, SHU9119 acts as an antagonist for MC4R but an agonist for MC1R. Structural analysis of the SHU9119-bound MC1R−G_s complex reveals that the bulky aromatic side-chain of D-Nal in SHU9119 pushes the side-chain of $L133^{3.36}$ of MC4R downward. As a result, it moved in front of $W258^{6.48}$, eliminating the capacity for TM6 outward movement. This $X^{3.36}$−$W^{6.48}$ molecular switch phenomenon has also been verified in MC3R and MC5R, demonstrating its conservation across the MCR family[111].

Subsequent active-state cryo-EM structures expanded the spectrum of ligand-bound conformations. Multiple complexes with α-MSH, afamelanotide, bremelanotide and setmelanotide showed a shared peptide-binding mode characterized by a U-shaped conformation inserted deeply into the orthosteric pocket, supported by extensive contacts involving conserved H−F−R−W motifs. These structures present hallmark features of GPCR activation such as outward movement of TM6, rearrangement of the $C^{6.47}$−$W^{6.48}$−$X^{6.49}$−$P^{6.50}$ toggle switch, and stabilization of the $N^{7.49}$−$P^{7.50}$−$X^{7.51}$−$X^{7.52}$−$Y^{7.53}$ motif. Collectively, these structures illuminate the general principles governing MC4R engagement of G_s and the resulting cAMP signaling.

Parallel efforts to characterize alternative ligand classes provided further mechanistic diversity. The structure of MC4R bound to the full agonist nanobody pN162 revealed that non-peptidic orthosteric ligands can occupy the same aromatic pocket normally engaged by peptide residues Phe or (D)Phe without relying on Ca^{2+} coordination[49]. Despite lacking the conserved H−X−R−W motif, the CDR3 loop of pN162 inserts deeply into the orthosteric pocket and mimics key interactions essential for receptor activation, while reproducing the core TM5/TM6 rearrangements observed in peptide-bound active states. These findings demonstrate the structural flexibility of MC4R in accommodating chemically diverse agonists while preserving a unified activation mechanism.

Structural investigation of small-molecule agonists added another dimension to receptor pharmacology. Structures of MC4R with chemically distinct small molecules demonstrated multiple hydrophobic subpockets within the orthosteric cavity, each capable of influencing ligand selectivity and signaling properties. A particularly informative example involves the classical small-molecule agonist THIQ. Unlike peptide ligands, THIQ inserts deeply into the hydrophobic core of the MC4R orthosteric pocket, forming extensive van der Waals interactions with MC4R-specific residues[50] such as $I129^{3.32}$, $F201^{5.47}$, and $I251^{6.51}$. These residues differ substantially from those found in homologous melanocortin receptors, thereby enabling THIQ to achieve high subtype selectivity. The tricyclic scaffold of THIQ is geometrically complementary to a hydrophobic subpocket unique to MC4R, stabilizing the outward position of TM6 and promoting a G_s-coupled active conformation. These structural observations explain both the potency and MC4R selectivity of THIQ and highlight how small molecules can exploit subtype-specific microenvironments for targeted receptor modulation.

Together, these diverse structures, spanning inactive, peptide-bound, nanobody-bound, and small-molecule-bound states, offer an increasingly unified view of MC4R conformational dynamics. Comparative analyses across these structures clarified how chemically distinct ligands stabilize different receptor states, modulate Ca^{2+} coordination, control access to hydrophobic subpockets, and shape the intracellular geometry for G-protein engagement. Despite these advances, a critical gap persisted: how MC4R engages $G_{q/11}$, the principal pathway mediating appetite suppression. Until recently, all available active-state structures featured G_s coupling, leaving the molecular signature of G_q-biased activation unresolved.

Multiple studies indicated that activating the MC4R-G_q pathway maintains negative energy balance without increasing cardiovascular risks[68] (Fig. 3A). The partially G_q-biased mechanism of setmelanotide has been suggested but remains to be structurally validated and elucidated. To fill this gap, we determined the cryo-EM structure of the setmelanotide−MC4R−G_q complex at 3.11 Å resolution, applying local refinement to enhance the density of TMs, yielding a final composed map for modeling (Fig. 3, Supporting Information Fig. S1 and Table S1). To stabilize the protein, we employed the NanoBiT tethering method to construct the complex as described previously[111], and an engineered $G\alpha_q$ chimera based on the mini-$G\alpha_{s/q}$71 scaffold[112,113] (Supporting Information Fig. S2). The complex was efficiently assembled on the membrane by co-expressing MC4R with $G\alpha_q$, $G\beta1$, and $G\gamma2$ subunits.

Structural comparison showed that the receptor portions of the setmelanotide−MC4R−G_q complex exhibit overall conformational similarity to those in the setmelanotide−MC4R−G_s (PDB: 7PIU)[51] and α-MSH−MC4R−G_s (PDB: 7F53)[50] complexes with Cα root mean square deviation (RMSD) values of 0.57 Å and 0.58 Å, respectively (Fig. 4). The ligand poses in the three complexes are similar and display a U-shaped structure inserted into the orthosteric pocket of the receptor (Fig. 4A).

The most notable difference lies in the binding pose of the first residue, R^3 (superscript indicates the peptide position number based on α-MSH) of setmelanotide, which forms a strong hydrogen bond with $N108^{ECL1}$ in the setmelanotide−MC4R−G_q structure, whereas in the setmelanotide−MC4R−G_s complex it instead engages $D122^{3.25}$ (Fig. 4B). In the α-MSH−MC4R−G_s structure, the spatially equivalent position is M^4, which inserts into a hydrophobic pocket formed by $V103^{2.63}$, $I104^{2.64}$, and $D122^{3.25}$. The positive charge of R^3 in setmelanotide (spatially equivalent to M^4 in α-MSH) prevents its insertion into the hydrophobic pocket of MC4R, instead prompting its shift toward a more polar environment and interactions with $N108^{ECL1}$ or $D122^{3.25}$ of MC4R (Fig. 4C). Accordingly, retaining a positively charged non-natural amino acid with optimized side-chain lengths may help to obtain lead compounds with stronger G_q signaling bias.

Despite the overall similarity in receptor architecture, the G protein regions of the setmelanotide−MC4R−G_q and setmelanotide−MC4R−G_s complexes show pronounced conformational divergence, as indicated by a Cα RMSD of 0.92 Å, underscoring a unique binding mode for G_q (Fig. 4A). Structural analysis revealed that the residues on Gα5 in the setmelanotide−MC4R−G_q complex undergo significant rearrangements of the polar network compared to the setmelanotide−MC4R−G_s complex. Specifically, the polar residue $Q370^{G\alpha5}$ in $G\alpha_s$ forms a strong hydrogen bond with $H222^{5.68}$, whereas the corresponding hydrophobic residue $L351^{G\alpha5}$ in $G\alpha_q$, owing to its hydrophobic side-chain, is unable to support this interaction (Fig. 4D). Instead, $H222^{5.68}$ in the $G\alpha_q$-bound MC4R undergoes a 2.5 Å displacement relative to its position in the $G\alpha_s$-bound receptor (quantified by the shift in the N atom of its imidazolyl moiety), thereby enabling the formation of a new hydrogen bond with $Q352^{G\alpha5}$ of $G\alpha_q$ (Fig. 4D). Meanwhile, at the equivalent position in $G\alpha_s$, the longer side-chain of the basic residue $R371^{G\alpha5}$ forms a stable hydrogen bond with $Y346^{G\alpha}$ of the β6 sheet, as well as a π−cation

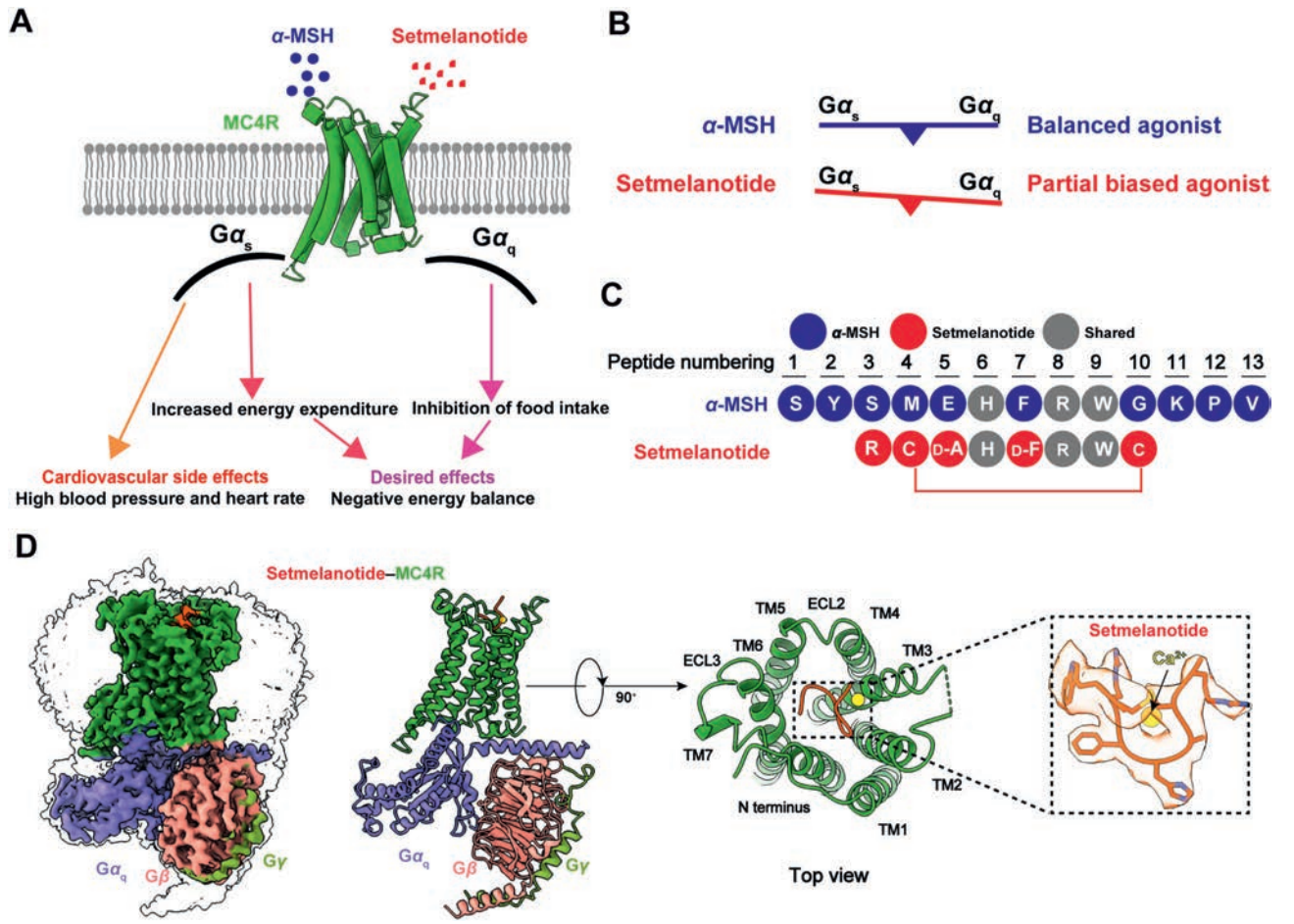

Figure 3 Cryo-EM structure of setmelanotide-bound MC4R in complex with G_q. (A) Schematic representation of the activation of different Gα pathways through MC4R, leading to distinct effects. (B) Schematic representation of the G protein bias of α-MSH and setmelanotide. (C) Amino acid sequences of α-MSH and setmelanotide. Residues are colored according to sequence conservation between α-MSH and setmelanotide. (D) Cryo-EM density map and cartoon representation of the setmelanotide−MC4R−G_q complex. Setmelanotide is shown in orange red, MC4R in forest green, calcium ion in yellow, Gα_q in purple, Gβ in salmon, and Gγ in olive drab.

interaction with $Y344^{G\alpha}$ of the $\beta6-\alpha4$ loop (Fig. 4D). Furthermore, to compensate for the reduced interaction between Gα5 and the β6 sheet in Gα_q, $D348^{G\alpha5}$ in Gα_q engages $Y327^{G\alpha}$ of the β6 sheet, whereas the corresponding residue $D367^{G\alpha5}$ in Gα_s instead forms a hydrogen bond with $R225^{5.71}$ on TM5 of MC4R (Fig. 4D). These rearrangements within the hydrogen-bonding network diminish the interaction between Gα_q and TM5 relative to Gα_s, resulting in a 1.9 Å outward displacement of TM5 (Fig. 4A). Additionally, we observed that $H158^{ICL2}$ of MC4R interacts with $H373^{G\alpha5}$ of Gα_s through a water-mediated hydrogen-bonding network. In Gα_q, however, the corresponding residue is $N354^{G\alpha5}$, a shorter and less polar side-chain that lacks associated water density and therefore does not support such an interaction (Fig. 4E). This indicates that allosteric ligands designed to bind at the MC4R−Gα interface and selectively stabilize Gα_q-favored interactions, while destabilizing those supporting Gα_s engagement, could represent a viable strategy to enhance Gα_q bias. This concept is supported by prior reports of GPCR allosteric modulators that tune G protein selectivity and downstream signaling bias by targeting the receptor−G protein interface[114-117].

Clearly, these differences in the binding modes of setmelanotide−MC4R complexes with distinct G proteins disclose the structural features that characterize the interactions of Gα_q and Gα_s with MC4R. As the first structural visualization of MC4R bound to G_q, setmelanotide−MC4R−G_q complex establishes essential molecular principles for understanding G-protein selectivity and biased agonism. This structure completes the current spectrum of MC4R conformational states and enables detailed comparison across G_s, G_q-specific coupling modes.

As structural and functional studies continue to advance, the mechanistic basis of biased MC4R signaling has become increasingly clear. High-resolution cryo-EM structures exhibit distinct conformational rearrangements of the transmembrane helices, intracellular loops, and G protein-binding interfaces that correspond to G_s- *versus* G_q-biased receptor states. These structural signatures not only elucidate the molecular determinants of ligand bias but also provide actionable templates for rational drug design. The ability to precisely target anorectic G_q pathways while avoiding G_s-associated cardiovascular activation represents a major conceptual and therapeutic breakthrough, one that underlies the success of drug candidates such as setmelanotide and guides the development of next-generation MC4R agonists and allosteric modulators.

5. Clinical translation

The translation of MC4R biology into clinical practice has followed a long and non-linear trajectory, marked by early optimism,

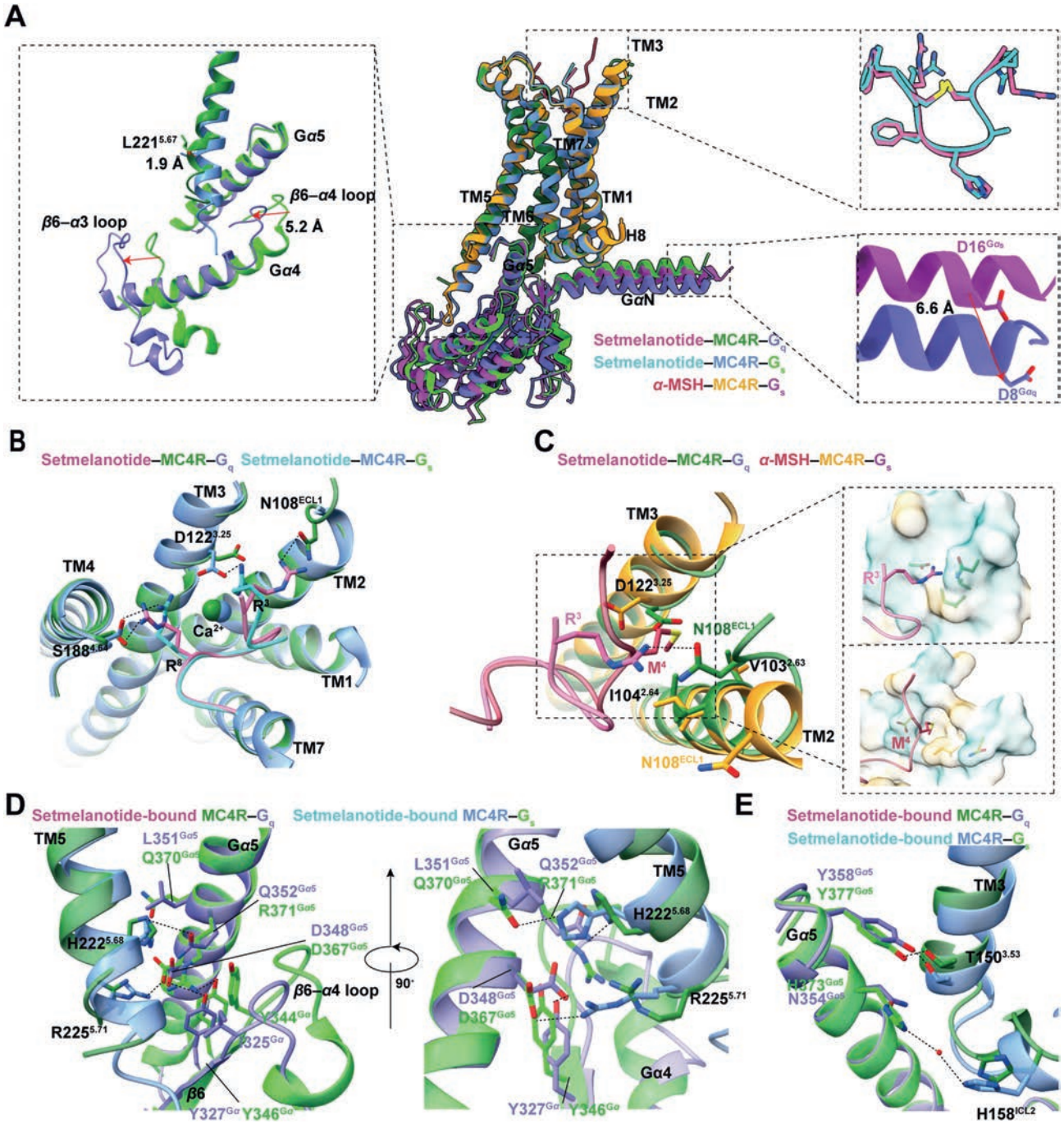

Figure 4 Analysis of setmelanotide binding and G protein coupling with MC4R. (A) Structural comparison of setmelanotide-bound MC4R−G_s, MC4R−G_q, and α-MSH-bound MC4R−G_s complexes. The notable structural differences in the orthosteric pocket and Gα conformation are highlighted in enlarged views. (B) Key amino acid position differences of ligands in the orthosteric pocket between setmelanotide−MC4R−G_q and MC4R−G_s complex. (C) Surrounding environment of R^3 and M^4 in setmelanotide−MC4R−G_q and α-MSH−MC4R−G_s. The results are shown with coloring on the molecular surface, ranging from dark cyan (most hydrophilic) to white to dark goldenrod (most lipophilic). Structural overlay comparison of setmelanotide-bound MC4R−G_q and α-MSH-bound MC4R−G_s showing the conformational differences between Gα5 and TM5 (D), as well as ICL2 (E). The key hydrogen bonds are shown as black dashed lines.

subsequent stagnation, and a resurgence driven by mechanistic and structural breakthroughs. The classical understanding of MC4R as a regulator of appetite and energy expenditure provided an attractive rationale for targeting the receptor[12,21,22,118]. However, the limitations of early drug candidates, most notably their cardiovascular liabilities and broad-spectrum agonist effects, exposed the danger of relying on incomplete mechanistic frameworks[119-121]. The eventual success of setmelanotide, more than two decades after MC4R was first linked to monogenic obesity, reflects the integration of genetic, physiological, structural, and pharmacological insights into a coherent therapeutic strategy. This section examines the clinical implications of MC4R signaling, the

development of therapeutic candidates, and the prospects for expanding MC4R-targeted therapies to broader metabolic conditions, with representative ligands targeting MC4R summarized in Table 2[36,48,49,55,56,114,122-141].

Physiologically, the half-life of endogenous α-MSH is only a few minutes, which is relatively short[142]. Consequently, the initial goal of melanocortin drug development was to enhance peptide stability and achieve selectivity for specific MCR subtypes. The main strategy involved modifying the amino acid sequence of endogenous melanocortin peptides based on their primary structures[57]. A commonly used and relatively successful modification was to take the 13 amino acid α-MSH peptide as the template (sequence: Ac-S^1-Y^2-S^3-M^4-E^5-H^6-F^7-R^8-W^9-G^{10}-K^{11}-P^{12}-V^{13}-NH_2), replace the fourth residue methionine with the more hydrophobic non-natural amino acid norleucine (Nle), and convert the seventh residue L-phenylalanine to the D-isomer, yielding [Nle^4-D-Phe^7]-MSH (also known as NDP-MSH/melanotan I/afamelanotide; sequence: Ac-S^1-Y^2-S^3-Nle^4-E^5-H^6-(D)F^7-R^8-W^9-G^{10}-K^{11}-P^{12}-V^{13}-NH_2). These modifications increased ligand stability while maintaining a potency comparable to α-MSH. Melanotan I acts as an agonist at all MCR subtypes except MC2R with nano- to sub-nanomolar potency. In 2014, melanotan I was approved in Europe for the treatment of erythropoietic protoporphyria in adults[123,124,143]. A similar strategy was applied to γ-MSH, in which the eighth tryptophan residue was converted to the D-isomer, generating D-Trp^8-γ-MSH. These two peptide ligands, built on amino acid substitutions, have long been used as research tools in the melanocortin system and are still widely employed today[144,145]. Subsequent work on melanotan I led to cyclization and sequence truncation, resulting in the synthesis of the eight-membered cyclic peptide melanotan II (sequence: Ac-Nle-c[D^1-H^2-(D)F^3-R^4-W^5-K^6]-NH_2). Melanotan II is a non-selective MCR agonist with nano- to sub-nanomolar potency across MCR subtypes[134]. Since its discovery, melanotan II has been used as a probe both *in vitro* and *in vivo*, and intracerebroventricular administration of melanotan II has been shown to suppress food intake in mice[146]. Interestingly, when D-Phe in melanotan II is replaced by 3-(2-naphthyl)-D-alanine (D-Nal(2′)), the resulting

Table 2 Representative ligands targeting MC4R: pharmacological characteristics and therapeutic development status

Ligand	Type	Signaling bias	Therapeutic indication	Stage	Ref.
Acthar Gel (ACTH preparation)	Peptide mixture	Non-selective MCR agonist	Infantile spasms, multiple sclerosis exacerbations, rheumatoid arthritis, lupus, nephrotic syndrome (primarily *via* MC2R activation)	Approved	56,114
ACTH (1−24)	Peptide	Non-selective MCR agonist	Diagnosis of adrenal insufficiency; inflammatory diseases (mainly *via* MC2R activation)	Approved	122
Afamelanotide	Peptide	Non-selective MCR agonist	Erythropoietic protoporphyria (*via* MC1R activation)	Approved	123,124
Bremelanotide	Peptide	Non-selective MCR agonist	Hypoactive sexual desire disorder (*via* MC4R activation)	Approved	125,126
Setmelanotide	Cyclic peptide	G_q-biased non-selective MCR agonist	Monogenic obesity	Approved	55
MK-0493	Small molecule	Partial selective MC4R agonist	Obesity	Phase II	127
Bivamelagon (LB54640)	Small molecule	MC4R agonist	Hypothalamic obesity	Phase II	128
LY2112688	Cyclic peptide	Non-selective MCR agonist	Obesity	Phase I	36
MC4-NN-0453	Cyclic peptide	Non-selective MCR agonist	Obesity	Phase I	129
AZD2820	Cyclic peptide	Non-selective MCR agonist	Obesity	Phase I	130
RM-718	Peptide	MC4R agonist	MC4R pathway impairment	Phase I	131
PF-07258669	Small molecule	MC4R antagonist	Obesity and metabolic disorders	Phase I	132
TCMCB-07	Cyclic peptide	MC3R/MC4R antagonist	Cachexia and weight loss disorders	Phase I	133
Melanotan II	Cyclic peptide	Non-selective MCR agonist	Obesity and metabolic disorders	Preclinical	134
RO27-3225	Peptide	Selective MC4R agonist	Appetite regulation and neuroinflammation	Preclinical	135
PF-00446687	Small molecule	Selective MC4R agonist	Appetite regulation and sexual dysfunction	Preclinical	136
pN162	Nanobody	MC3R/MC4R agonist	Appetite regulation	Preclinical	49
THIQ	Small molecule	Selective MC4R agonist	Obesity	Preclinical	137
SHU9119	Cyclic peptide	MC3R/MC4R antagonist; partial agonist at MC1R/MC5R	Cancer cachexia	Preclinical	138
PG-901	Cyclic peptide	MC3R/MC4R antagonist; full agonist at MC1R/MC5R	Cachexia and appetite disorders	Preclinical	139
PG-934	Cyclic peptide	MC3R/MC4R antagonist	Cancer cachexia	Preclinical	48
SBL-MC-31	Cyclic peptide	Selective MC4R antagonist	Cancer cachexia	Preclinical	48
Ipsen 5i	Small molecule	MC4R inverse agonist with MAPK pathway agonism	Rescue of trafficking-defective MC4R mutants	Preclinical	140
KAF2039-7	Cyclic peptide	MC4R inverse agonist	Potential therapy for cachexia, anorexia, and growth disorders	Preclinical	141

peptide, SHU9119, partially retains agonist activity at MC1R and MC5R but antagonizes MC3R and MC4R[138]. As the first peptide ligand with strong antagonistic activity at both MC3R and MC4R, SHU9119 was shown to markedly increase food intake when administered intracerebroventricularly in mice[147]. Building on SHU9119, the first highly selective full agonist for MC5R, PG-901 (sequence: Ac-Nle-c[D^1-P^2-D^3-F^4-R^5-W^6-K^7]-NH_2), was developed; this peptide retains antagonistic activity at MC3R and MC4R[139].

AgRP is an inverse agonist of MC4R, and drug development efforts targeting AgRP have mainly focused on the treatment of negative energy balance conditions such as anorexia, cachexia, and growth failure in children. For example, a patient with cachexia was reported to carry an A67T mutation in AgRP[148]. Structural studies have shown that the key "RFF" motif responsible for AgRP activity is located within an exposed β-hairpin loop[149]. A macrocyclic octapeptide scaffold derived from AgRP, c[P^1-R^2-F^3-F^4-N^5-A^6-Nle^7-(D)P^8], can markedly increase food intake in mice and reduces MC4R-mediated cAMP production below basal levels[141].

Several MCR-targeted drugs derived from amino acid-modified peptide ligands have already reached the market. Acthar Gel was the first melanocortin-based drug approved for use in humans, entering the market in the early 1950s, roughly 40 years before the discovery of MCRs[56,58]. Acthar Gel, also known as repository corticotropin injection (RCI), is prepared from porcine pituitary extracts and therefore contains not only ACTH as the main component but also other pituitary peptides, although detailed information regarding these additional peptides and their potential actions has not been disclosed. Currently, Acthar Gel is approved for 19 different indications, including infantile spasms, acute exacerbations of multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, serum sickness, sarcoidosis, and nephrotic syndrome, among others[150]. In addition, ACTH(1−24) has been approved following clinical trials for the diagnosis of adrenal insufficiency and the treatment of inflammatory diseases[122]. However, adrenal side-effects mediated by MC2R activation limit the long-term use of ACTH. The development of novel ACTH-based agents that lack adrenal adverse events may represent an ideal strategy.

In 2014, afamelanotide was approved for marketing. This drug induces the production of eumelanin in the skin, thereby increasing tolerance to sunlight and artificial light and preventing the characteristic phototoxic reactions experienced by patients with erythropoietic protoporphyria (EPP)[151]. Moreover, studies have shown that afamelanotide, through activation of MC1R, can modulate DNA damage-repair mechanisms and thus may hold considerable potential for the prevention of skin cancers and other disorders associated with defective DNA repair[152,153]. Bremelanotide, a deaminated derivative of melanotan II that may represent a metabolite of melanotan II in humans, was approved in 2019. Multiple studies have indicated that its therapeutic effect in enhancing sexual desire in women with hypoactive sexual desire disorder is primarily mediated *via* MC4R activation[125,126].

The development of drugs targeting neuronal MC4R for the treatment of obesity has long been a major focus within this receptor family. Several oral small molecules and injectable peptide agonists have been clinically evaluated for obesity treatment, including the oral small-molecule MK-0493 (Merck)[127], bivamelagon (Rhythm Pharmaceuticals)[128], PF-07258669 (Pfizer)[132] and the peptide agonists LY2112688 (Eli Lilly)[36], MC4-NN-0453 (Novo)[129], RM-718 (Rhythm Pharmaceuticals)[131], AZD2820 (AstraZeneca)[130], and setmelanotide (Rhythm Pharmaceuticals)[55]. However, despite demonstrating preclinical efficacy in obesity models, most MC4R agonist candidates developed as potential anti-obesity therapies have ultimately failed due to insufficient clinical efficacy and significant adverse effects. To date, setmelanotide is the only agent in this class that has successfully reached the market. Setmelanotide is a cyclic octapeptide with high potency at the two neuronal MCRs (EC_{50} = 0.032 nmol/L at MC4R and 0.69 nmol/L at MC3R)[154]. In 2020, setmelanotide received FDA approval for the treatment of obesity caused by LoF variants in the genes encoding proopiomelanocortin (*POMC*), the leptin receptor (*LEPR*), or proprotein convertase subtilisin/kexin type 1 (*PCSK1*)[54]. In addition, compared with other MC4R agonists evaluated clinically, setmelanotide does not induce cardiovascular adverse events[118,155]. The relative safety of setmelanotide may stem from its strong efficacy in engaging G_q-dependent signaling[156]. MC4R-mediated G_q signaling has been shown to reduce food intake without affecting cardiovascular parameters[44]. Thus, MC4R-biased ligands that selectively activate G_q signaling may represent superior candidate drugs for the treatment of obesity.

At present, all five marketed MCR-targeted drugs function as non-selective agonists across MCR subtypes. While they exert therapeutic effects *via* one subtype, concomitant activation of other subtypes may lead to a range of adverse effects. For instance, although setmelanotide exerts anti-obesity effects through MC4R activation, approximately 50% of treated patients develop skin hyperpigmentation and darkening due to activation of MC1R in melanocytes[157]. This highlights the distinct physiological roles of different MCRs: MC1R primarily regulates skin pigmentation, MC2R mediates adrenal steroidogenesis, and MC5R is largely involved in peripheral processes like lipid metabolism and exocrine gland secretion, making them less directly aligned with the central appetite and energy-expenditure control mediated by MC4R. In contrast, MC3R participates in hypothalamic circuits regulating nutrient partitioning, metabolic efficiency, and circadian feeding rhythms, suggesting a potential complementary role with MC4R in energy homeostasis. Preclinical studies indicate that co-activation of MC3R and MC4R may produce greater metabolic benefits than selective MC4R activation alone. However, robust clinical evidence for this strategy remains limited, and the physiological consequences of MC3R activation are not fully understood. Therefore, the development of subtype-selective agents has remained a key objective in drug discovery targeting MC4R.

MC4R has received particular attention from the pharmaceutical industry because of its critical role in obesity. In 2002, the first selective small-molecule MC4R agonist with nanomolar potency, THIQ, was reported[137]. THIQ is a tetrahydroisoquinoline-based ligand and was the first small-molecule agonist with high affinity (IC_{50} = 1.2 nmol/L) and potency (EC_{50} = 2.1 nmol/L) at MC4R[137]. Structure−activity relationship studies demonstrated that THIQ competes with NDP-MSH for binding to MC4R, indicating that it occupies the orthosteric site, while at the same time displaying a unique pharmacophore[158]. Because MC4R agonists reduce food intake in rodents, small-molecule MC4R agonists were considered potential therapeutics for weight loss. However, THIQ, despite its selectivity for MC4R, still produced adverse effects commonly observed with activation of other MCR subtypes, including hypertension, tachycardia, skin darkening, and increased sexual arousal[159-161]. To mitigate such side-effects, MC3R, which is also expressed in the central nervous system and

involved in the regulation of food intake and energy expenditure, has emerged as an attractive alternative target[147].

Ipsen 5i, a representative member of the aminobenzimidazole series developed by Roubert and colleagues at Ipsen, exhibits an affinity of 2 nmol/L for human MC4R[140]. Ipsen 5i is a high-affinity inverse agonist at MC4R and competes with NDP-MSH for receptor binding, but it shows much lower affinity for MC3R (K_i = 400 nmol/L)[162]. Although Ipsen 5i reduces basal signaling through the classical G_s—cAMP pathway, it retains agonist activity on the mitogen-activated protein kinase (MAPK) pathway[163]. LoF mutations in MC4R cause severe early-onset obesity in humans, and functional studies have shown that most of these mutants are nonfunctional because they are retained intracellularly and fail to traffic to the plasma membrane. One study demonstrated that Ipsen 5i can rescue the trafficking and signaling of the majority (73%) of intracellularly retained MC4R mutants[164].

MK-0493 is a piperazinyl urea-based small-molecule compound. It is an effective, orally active, and selective partial agonist of MC4R. In preclinical animal models, oral administration of MK-0493 produced robust weight-reducing effects, indicating that the compound can reach hypothalamic target sites, and it did not induce side-effects such as increased erectile responses[165]. However, in a clinical study, although fixed doses of MK-0493 induced modest weight loss compared with controls during the first 12 weeks, no statistically significant additional weight loss was observed between weeks 12 and 18[127]. Taken together, these findings suggest that MK-0493 is unlikely to be a viable candidate for the development of anti-obesity therapies.

Increasing attention is now focused on whether MC4R agonists may benefit individuals with polygenic or common forms of obesity, a far larger patient population. A growing body of evidence indicates that even partial impairment of the melanocortin pathway, arising from polygenic susceptibility, obesogenic environments, or neuroendocrine dysregulation, may render individuals more responsive to therapies that selectively enhance MC4R activity[109]. Early exploratory studies suggest that some patients with heterozygous MC4R variants or with functional impairments in upstream POMC pathways may show meaningful weight loss on setmelanotide, though results vary[27,166,167]. Larger clinical trials are underway to clarify the extent to which MC4R agonism can benefit broader populations beyond monogenic obesity syndromes.

A particularly promising direction involves combination therapies. The success of GLP-1R agonists and dual/triple incretin agonists has ignited interest in combining agents that target distinct aspects of energy balance[168]. Mechanistic studies support a functional interplay between GLP-1 and melanocortin signaling at multiple levels. GLP-1R is expressed in arcuate POMC neurons, where GLP-1 or exendin-4 directly depolarizes these neurons and increases firing rate—an effect blocked by GLP-1R antagonism[169]. This activation promotes α-MSH release and subsequent engagement of MC4R-expressing neurons in the PVN, establishing a circuit-level mechanism by which GLP-1 signaling recruits the melanocortin system to suppress food intake[169]. Consistent with this, the anorectic and weight-loss effects of liraglutide are attenuated by central administration of the MC3/4R antagonist SHU9119 and enhanced by the agonist MTII[170]. This crosstalk extends beyond central circuits. MC4R expressed on enteroendocrine L cells directly stimulates GLP-1 and peptide YY (PYY) secretion upon activation; acute melanocortin peptide administration increases circulating GLP-1 levels in mice, an effect confirmed as MC4R-dependent in *ex vivo* intestinal preparations[171,172].

Preclinical and early clinical studies indicate that co-activating GLP-1R and MC4R produces additive or synergistic metabolic benefits[74,173]. In diet-induced obese rodents, combination therapy with liraglutide and setmelanotide induced greater anorexia and metabolic improvement than either monotherapy[31]. A more recent approach engineered a monomeric dual GLP-1R/MC4R agonist (KCEM1) based on exendin-4 and α-MSH; daily administration in obese rats achieved body weight reductions comparable to semaglutide and tirzepatide, with superior glycemic control[74]. Clinically, patients previously treated with GLP-1R agonists or tirzepatide showed enhanced responses to subsequent bremelanotide treatment, and early-stage trials are evaluating whether MC4R activation can extend incretin-based weight loss (ClinicalTrials.gov identifier: NCT06565611)[173]. These observations position MC4R as a rational target for combination regimens, particularly for patients experiencing efficacy plateaus with GLP-1RAs. Actually, MC4R integrates multiple neuroendocrine signals, thereby governing energy homeostasis. MC4R activation enhances leptin signaling in LEPR-deficient obesity[174] and interacts with 5-hydroxytryptamine receptor 2C (5-HT_{2C}R), which regulates POMC neuronal activity, to modulate food intake[175]. Co-activation of MC3R and MC4R has also been proposed to improve metabolic regulation, given MC3R's role in nutrient partitioning *versus* MC4R's direct effects on appetite and energy expenditure. However, while preclinical studies—including nonhuman primate data—suggest potential benefits, robust clinical evidence for MC3R/MC4R dual agonism remains elusive. Nonetheless, structure-guided MC4R agonists with improved selectivity and reduced off-target activity may prove suitable for chronic coadministration with incretin analogues. There is also emerging evidence implicating MC4R in neuropsychiatric conditions such as addictive behavior, stress resilience, and mood regulation, though the translational potential in these domains remains to be fully explored.

Beyond obesity, MC4R signaling influences multiple physiological aspects with potential therapeutic relevance. The receptor modulates autonomic tone[176,177], glucose metabolism[13], inflammation[17], reward circuits[178], and neuroendocrine axes[179]. Preclinical studies show that MC4R activation can improve hepatic insulin sensitivity, increase GLP-1 secretion from enteroendocrine L cells, and reduce steatosis in metabolic disease models[171]. These findings suggest the possibility of MC4R agonists in managing type 2 diabetes, metabolic dysfunction-associated steatotic liver disease (MASLD), or metabolic dysfunction-associated obesity without the cardiovascular risks typical of past agents.

6. Future directions

The rapid advances in MC4R biology, structural elucidation, and pathway-selective pharmacology have transformed the receptor from a once-challenging and clinically uncertain target into an important node in contemporary anti-obesity therapeutics. Looking ahead, several emerging research directions are poised to shape the next generation of MC4R-based therapies. These directions span fundamental mechanistic investigations, structure-enabled ligand design, combination therapies that integrate MC4R with other metabolic pathways, and translational strategies aimed at expanding therapeutic indications beyond monogenic obesity.

One important direction for future research is the design of MC4R ligands that combine both selectivity and biased signaling, in order to reduce the side-effects caused by broad activation of

MCRs and G_s signaling pathways. Such agonists could mimic or improve the naturally occurring biased signaling patterns observed *in vivo*. Although recent studies have demonstrated the therapeutic advantages of $G_{q/11}$-selective or G_q-biased agonism for appetite suppression, the complete catalog of MC4R's intracellular effectors is still evolving. Ion-channel modulation, β-arrestin scaffolding, and noncanonical intracellular partners are likely involved in cell-type-specific MC4R functions, but these mechanisms remain insufficiently characterized. Understanding how these mechanisms interact within different neuronal populations, such as PVN MC4R neurons *versus* autonomic circuits, will be key to designing ligands with tailored signaling profiles and improved safety margins.

Emerging therapeutic strategies will not only focus on selective MC4R activation but also embrace combinational approaches that integrate MC4R signaling with other metabolic pathways. The rise of GLP-1R agonists has transformed the global obesity treatment landscape and highlighted the potential for multi-pathway therapies that achieve deeper, more durable, and more physiologically aligned weight loss effects. MC4R agonists complement GLP-1 and glucose-dependent insulinotropic polypeptide (GIP) signaling at both the neurocircuit and molecular levels, offering opportunities for additive or synergistic effects on appetite regulation, metabolic efficiency, and glycemic control[74]. In the future, combination therapies targeting MC4R alongside incretin receptors, amylin receptors, or even peripheral metabolic targets could constitute a new generation of anti-obesity regimens capable of surpassing the efficacy plateau seen with monotherapies.

Beyond obesity, MC4R-targeted therapies may find applications in disorders characterized by dysregulated melanocortin signaling or impaired appetite control. These include hypothalamic obesity following craniopharyngioma or CNS injury[180], syndromic hyperphagia[55,181], cancer cachexia[182], lipodystrophy-associated metabolic dysfunction[183], and conditions involving impaired sympathetic output or autonomic imbalance[87,184]. Because MC4R influences a wide range of processes, such as glucose tolerance, linear growth, reproductive function, and autonomic regulation, careful mapping of its cell-type-specific functions could open new therapeutic domains beyond traditional metabolic diseases.

Mechanistically, numerous questions remain to be addressed. MC4R can undergo both homodimerization and heterodimerization with other MCR subtypes, and the number, types, and modes of G protein coupling associated with these dimeric complexes remain to be fully elucidated[185,186]. Melanocortin 2 receptor accessory protein 2 (MRAP2) is known to modulate MC4R activity, and dysfunction of MRAP2 can lead to obesity phenotypes resembling those caused by MC4R gene defects; however, the precise molecular mechanisms underlying this modulation are still unclear[187-192]. In addition, MC4R can interact with Kir7.1 in a G protein-independent manner, modulating neuronal excitation or inhibition by altering the transmembrane K^+ gradient. The structural and functional basis of this interaction, as well as the druggability of this noncanonical signaling axis, remain important areas for future investigation.

In summary, future research will build on the conceptual and structural foundations established over the past decade, moving toward a new era in which MC4R is targeted with pathway selectivity, structural precision, and combinational strategy. As obesity becomes one of the most pressing global health challenges and as incretin-based treatment reshapes the therapeutic landscape, MC4R-targeted approaches will be increasingly important for expanding therapy options, improving clinical outcomes, and unlocking a broader spectrum of metabolic benefits. The convergence of structural biology, biased pharmacology, and combinational therapeutics positions MC4R at the forefront of next-generation anti-obesity drug development.

Data and materials availability

The atomic coordinate and cryo-EM density maps of the setmelanotide−MC4R−G_q complex have been deposited in the Protein Data Bank (PDB) under accession code 21LT and in the Electron Microscopy Data Bank (EMDB) under accession codes 67804 (main map), 67801 (consensus map), and 67803 (local refinement map), respectively. All relevant data are available from the authors and/or included in the manuscript or Supporting Information.

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Author contributions

Wenbo Feng performed experiments, retrieved the related literature, wrote the draft, and created images and tables. Ming-Wei Wang conceived the study and revised the manuscript with Qingtong Zhou. All authors have read and approved this version of the manuscript.

Conflicts of interest

All the authors declare no conflicts of interest.

Appendix A. Supporting information

Supporting information to this article can be found online at https://doi.org/10.1016/j.apsb.2026.06.035.

References

1. Moiz A, Filion KB, Tsoukas MA, Yu OHY, Peters TM, Eisenberg MJ. The expanding role of GLP-1 receptor agonists: a narrative review of current evidence and future directions. *eClinicalMedicine* 2025;**86**:103363.
2. Guo HY, Yang J, Huang JH, Xu L, Lv YH, Wang YX, et al. Comparative efficacy and safety of GLP-1 receptor agonists for weight reduction: a model-based meta-analysis of placebo-controlled trials. *Obes Pillars* 2025;**13**:100162.
3. Zhou QT, Li GY, Hang KN, Li J, Yang DH, Wang MW. Weight loss blockbuster development: a role for unimolecular polypharmacology. *Annu Rev Pharmacol Toxicol* 2025;**65**:191−213.
4. Zhou QT, Zhao FH, Zhang Y, Yang DH, Wang MW. Structural pharmacology and mechanisms of GLP-1R signaling. *Trends Pharmacol Sci* 2025;**46**:422−36.

5. Zheng ZK, Zong Y, Ma YY, Tian YC, Pang YD, Zhang CQ, et al. Glucagon-like peptide-1 receptor: mechanisms and advances in therapy. *Signal Transduct Target Ther* 2024;**9**:234.
6. Berg S, Stickle H, Rose SJ, Nemec EC. Discontinuing glucagon-like peptide-1 receptor agonists and body habitus: a systematic review and meta-analysis. *Obes Rev* 2025;**26**:e13929.
7. Wilding JPH, Batterham RL, Calanna S, Davies M, Van Gaal LF, Lingvay I, et al. Once-weekly semaglutide in adults with overweight or obesity. *N Engl J Med* 2021;**384**:989−1002.
8. Look M, Dunn JP, Kushner RF, Cao D, Harris C, Gibble TH, et al. Body composition changes during weight reduction with tirzepatide in the SURMOUNT-1 study of adults with obesity or overweight. *Diabetes Obes Metab* 2025;**27**:2720−9.
9. Fatima MT, Ahmed I, Fakhro KA, Akil ASA. Melanocortin-4 receptor complexity in energy homeostasis, obesity and drug development strategies. *Diabetes Obes Metab* 2022;**24**:583−98.
10. Yeo GSH, Chao DHM, Siegert AM, Koerperich ZM, Ericson MD, Simonds SE, et al. The melanocortin pathway and energy homeostasis: from discovery to obesity therapy. *Mol Metab* 2021;**48**: 101206.
11. Yang LK, Tao YX. Biased signaling at neural melanocortin receptors in regulation of energy homeostasis. *Biochim Biophys Acta Mol Basis Dis* 2017;**1863**:2486−95.
12. Krashes MJ, Lowell BB, Garfield AS. Melanocortin-4 receptor-regulated energy homeostasis. *Nat Neurosci* 2016;**19**:206−19.
13. Rossi J, Balthasar N, Olson D, Scott M, Berglund E, Lee CE, et al. Melanocortin-4 receptors expressed by cholinergic neurons regulate energy balance and glucose homeostasis. *Cell Metab* 2011;**13**: 195−204.
14. Do Carmo JM, Da Silva AA, Wang Z, Fang T, Aberdein N, Perez de Lara CE, et al. Role of the brain melanocortins in blood pressure regulation. *Biochim Biophys Acta Mol Basis Dis* 2017;**1863**: 2508−14.
15. Gantz I, Fong TM. The melanocortin system. *Am J Physiol Endocrinol Metab* 2003;**284**:E468−74.
16. Ji LQ, Hong Y, Tao YX. Melanocortin-5 receptor: pharmacology and its regulation of energy metabolism. *Int J Mol Sci* 2022;**23**:8727.
17. Wang W, Guo DY, Lin YJ, Tao YX. Melanocortin regulation of inflammation. *Front Endocrinol* 2019;**10**:683.
18. Roselli-Rehfuss L, Mountjoy KG, Robbins LS, Mortrud MT, Low MJ, Tatro JB, et al. Identification of a receptor for gamma melanotropin and other proopiomelanocortin peptides in the hypothalamus and limbic system. *Proc Natl Acad Sci U S A* 1993;**90**: 8856−60.
19. Lubrano-Berthelier C, Durand E, Dubern B, Shapiro A, Dazin P, Weill J, et al. Intracellular retention is a common characteristic of childhood obesity-associated MC4R mutations. *Hum Mol Genet* 2003;**12**:145−53.
20. Ong SG, Dehghan R, Dorajoo R, Liu JJ, Sng AA, Lee YS, et al. Novel melanocortin-3 and -4 receptor functional variants in Asian children with severe obesity. *J Clin Endocrinol Metab* 2024;**109**:e1249−59.
21. Huszar D, Lynch CA, Fairchild-Huntress V, Dunmore JH, Fang Q, Berkemeier LR, et al. Targeted disruption of the melanocortin-4 receptor results in obesity in mice. *Cell* 1997;**88**:131−41.
22. Kühnen P, Krude H, Biebermann H. Melanocortin-4 receptor signalling: importance for weight regulation and obesity treatment. *Trends Mol Med* 2019;**25**:136−48.
23. Lotta LA, Mokrosiński J, Mendes de Oliveira E, Li C, Sharp SJ, Luan J, et al. Human gain-of-function MC4R variants show signaling bias and protect against obesity. *Cell* 2019;**177**:597−607.e9.
24. Rojo D, McCarthy C, Raingo J, Rubinstein M. Mouse models for V103I and I251L gain of function variants of the human MC4R display decreased adiposity but are not protected against a hypercaloric diet. *Mol Metab* 2020;**42**:101077.
25. Tao YX. Melanocortin receptors. *Biochim Biophys Acta Mol Basis Dis* 2017;**1863**:2411−3.
26. Smith AI, Funder JW. Proopiomelanocortin processing in the pituitary, central nervous system, and peripheral tissues. *Endocr Rev* 1988;**9**:159−79.
27. Bouret SG. Developmental programming of hypothalamic melanocortin circuits. *Exp Mol Med* 2022;**54**:403−13.
28. De Solis AJ, Del Río-Martín A, Radermacher J, Chen W, Steuernagel L, Bauder CA, et al. Reciprocal activity of AgRP and POMC neurons governs coordinated control of feeding and metabolism. *Nat Metab* 2024;**6**:473−93.
29. Timper K, Brüning JC. Hypothalamic circuits regulating appetite and energy homeostasis: pathways to obesity. *Dis Model Mech* 2017;**10**: 679−89.
30. Chen A, Metzger J, Trumbauer M, Guan XM, Yu H, Frazier E, et al. Role of the melanocortin-4 receptor in metabolic rate and food intake in mice. *Transgenic Res* 2000;**9**:145−54.
31. Clemmensen C, Finan B, Fischer K, Tom RZ, Legutko B, Sehrer L, et al. Dual melanocortin-4 receptor and GLP-1 receptor agonism amplifies metabolic benefits in diet-induced obese mice. *EMBO Mol Med* 2015;**7**:288−98.
32. Shen Y, Fu WY, Cheng EY, Fu AK, Ip NY. Melanocortin-4 receptor regulates hippocampal synaptic plasticity through a protein kinase A-dependent mechanism. *J Neurosci* 2013;**33**:464−72.
33. Muceniece R, Zvejniece L, Vilskersts R, Liepinsh E, Baumane L, Kalvinsh I, et al. Functional evaluation of THIQ, a melanocortin 4 receptor agonist, in models of food intake and inflammation. *Basic Clin Pharmacol Toxicol* 2007;**101**:416−20.
34. Wessells H, Levine N, Hadley ME, Dorr R, Hruby V. Melanocortin receptor agonists, penile erection, and sexual motivation: human studies with Melanotan II. *Int J Impot Res* 2000;**12**:S74−9.
35. Kievit P, Halem H, Marks DL, Dong JZ, Glavas MM, Sinnayah P, et al. Chronic treatment with a melanocortin-4 receptor agonist causes weight loss, reduces insulin resistance, and improves cardiovascular function in diet-induced obese *Rhesus macaques*. *Diabetes* 2013;**62**:490−7.
36. Greenfield JR, Miller JW, Keogh JM, Henning E, Satterwhite JH, Cameron GS, et al. Modulation of blood pressure by central melanocortinergic pathways. *N Engl J Med* 2009;**360**:44−52.
37. Mark AL. Cardiovascular side effects of antiobesity drugs. *Circulation* 2009;**120**:719−21.
38. Israeli H, Degtjarik O, Fierro F, Chunilal V, Gill AK, Roth NJ, et al. Structure reveals the activation mechanism of the MC4 receptor to initiate satiation signaling. *Science* 2021;**372**:808−14.
39. Singh U, Jiang J, Saito K, Toth BA, Dickey JE, Rodeghiero SR, et al. Neuroanatomical organization and functional roles of PVN MC4R pathways in physiological and behavioral regulations. *Mol Metab* 2022;**55**:101401.
40. Ju SH, Cho GB, Sohn JW. Understanding melanocortin-4 receptor control of neuronal circuits: toward novel therapeutics for obesity syndrome. *Pharmacol Res* 2018;**129**:10−9.
41. Sharma S, Thibodeau S, Lytton J. Signal pathway analysis of selected obesity-associated melanocortin-4 receptor class V mutants. *Biochim Biophys Acta Mol Basis Dis* 2020;**1866**:165835.
42. Wei R, Li D, Jia S, Chen Y, Wang J. MC4R in central and peripheral systems. *Adv Biol (Weinh)* 2023;**7**:e2300035.
43. Metzger PJ, Zhang A, Carlson BA, Sun H, Cui Z, Li Y, et al. A human obesity-associated MC4R mutation with defective Gq/11α signaling leads to hyperphagia in mice. *J Clin Investig* 2024;**134**:e165418.
44. Li YQ, Shrestha Y, Pandey M, Chen M, Kablan A, Gavrilova O, et al. Gq/11α and Gsα mediate distinct physiological responses to central melanocortins. *J Clin Investig* 2015;**126**:40−9.
45. Deem JD, Faber CL, Morton GJ. AgRP neurons: regulators of feeding, energy expenditure, and behavior. *FEBS J* 2022;**289**:2362−81.
46. Joseph CG, Bauzo RM, Xiang Z, Shaw AM, Millard WJ, Haskell-Luevano C. Elongation studies of the human agouti-related protein (AGRP) core decapeptide (Yc[CRFFNAFC]Y) results in antagonism at the mouse melanocortin-3 receptor. *Peptides* 2003;**24**:263−70.

47. Nijenhuis WAJ, Oosterom J, Adan RAH. AgRP(83−132) acts as an inverse agonist on the human-melanocortin-4 receptor. *Mol Endocrinol* 2001;**15**:164−71.
48. Gimenez LE, Martin C, Yu J, Hollanders C, Hernandez CC, Wu Y, et al. Novel cocrystal structures of peptide antagonists bound to the human melanocortin receptor 4 unveil unexplored grounds for structure-based drug design. *J Med Chem* 2024;**67**:2690−711.
49. Fontaine T, Busch A, Laeremans T, De Cesco S, Liang YL, Jaakola VP, et al. Structure elucidation of a human melanocortin-4 receptor specific orthosteric nanobody agonist. *Nat Commun* 2024;**15**:7029.
50. Zhang H, Chen LN, Yang D, Mao C, Shen Q, Feng W, et al. Structural insights into ligand recognition and activation of the melanocortin-4 receptor. *Cell Res* 2021;**31**:1163−75.
51. Heyder NA, Kleinau G, Speck D, Schmidt A, Paisdzior S, Szczepek M, et al. Structures of active melanocortin-4 receptor-gs-protein complexes with NDP-alpha-MSH and setmelanotide. *Cell Res* 2021;**31**:1176−89.
52. Yu J, Gimenez LE, Hernandez CC, Wu Y, Wein AH, Han GW, et al. Determination of the melanocortin-4 receptor structure identifies Ca^{2+} as a cofactor for ligand binding. *Science* 2020;**368**:428−33.
53. Feng W, Zhou Q, Zheng C, Yang D, Wang MW. Structural basis for the constitutive activity of the melanocortin receptor family. *Structure* 2025;**33**:1074-87.e5.
54. Markham A. Setmelanotide: first approval. *Drugs* 2021;**81**:397−403.
55. Clément K, van den Akker E, Argente J, Bahm A, Chung WK, Connors H, et al. Efficacy and safety of setmelanotide, an MC4R agonist, in individuals with severe obesity due to LEPR or POMC deficiency: single-arm, open-label, multicentre, phase 3 trials. *Lancet Diabetes Endocrinol* 2020;**8**:960−70.
56. Hench PS, Kendall EC, Slocumb CH, Polley HF. Effects of cortisone acetate and pituitary ACTH on rheumatoid arthritis, rheumatic fever and certain other conditions. *Arch Intern Med* 1950;**85**:545−666.
57. Ashton H, Millman JE, Telford R, Thompson JW, Davies TF, Hall R, et al. Psychopharmacological and endocrinological effects of melanocyte stimulating hormones in normal man. *Psychopharmacology* 1977;**55**:165−72.
58. Mountjoy KG, Robbins LS, Mortrud MT, Cone RD. The cloning of a family of genes that encode the melanocortin receptors. *Science* 1992;**257**:1248−51.
59. Mountjoy KG, Mortrud MT, Low MJ, Simerly RB, Cone RD. Localization of the melanocortin-4 receptor (MC4-R) in neuroendocrine and autonomic control circuits in the brain. *Mol Endocrinol* 1994;**8**:1298−308.
60. Fathi Z, Iben LG, Parker EM. Cloning, expression, and tissue distribution of a fifth melanocortin receptor subtype. *Neurochem Res* 1995;**20**:107−13.
61. You P, Hu H, Chen Y, Zhao Y, Yang Y, Wang T, et al. Effects of melanocortin 3 and 4 receptor deficiency on energy homeostasis in rats. *Sci Rep* 2016;**6**:34938.
62. Salazar-Valencia IG, Villamil-Ramírez H, Barajas-Olmos F, Guevara-Cruz M, Macias-Kauffer LR, García-Ortiz H, et al. Effect of the melanocortin 4-receptor Ile269Asn mutation on weight loss response to dietary, phentermine and bariatric surgery interventions. *Genes* 2022;**13**:2267.
63. Vaisse C, Clement K, Durand E, Hercberg S, Guy-Grand B, Froguel P. Melanocortin-4 receptor mutations are a frequent and heterogeneous cause of morbid obesity. *J Clin Investig* 2000;**106**:253−62.
64. Olney JJ, Sprow GM, Navarro M, Thiele TE. The protective effects of the melanocortin receptor (MCR) agonist, melanotan-II (MTII), against binge-like ethanol drinking are facilitated by deletion of the MC3 receptor in mice. *Neuropeptides* 2014;**48**:47−51.
65. Vemulapalli R, Kurowski S, Salisbury B, Parker E, Davis H. Activation of central melanocortin receptors by MT-II increases cavernosal pressure in rabbits by the neuronal release of NO. *Br J Pharmacol* 2001;**134**:1705−10.
66. Patruno S, Garrido-Mesa J, Romano M, Perretti M, Montero-Melendez T. Ligand bias and its association with pro-resolving actions of melanocortin drugs. *Front Pharmacol* 2018;**9**:919.
67. Gonçalves JPL, Palmer D, Meldal M. MC4R agonists: structural overview on antiobesity therapeutics. *Trends Pharmacol Sci* 2018;**39**:402−23.
68. Liu Z, Hruby VJ. MC4R biased signalling and the conformational basis of biological function selections. *J Cell Mol Med* 2022;**26**:4125−36.
69. Breit A, Büch TR, Boekhoff I, Solinski HJ, Damm E, Gudermann T. Alternative G protein coupling and biased agonism: new insights into melanocortin-4 receptor signalling. *Mol Cell Endocrinol* 2011;**331**:232−40.
70. Yao H, Zhang A, Li D, Wu Y, Wang CZ, Wan JY, et al. Comparative effectiveness of GLP-1 receptor agonists on glycaemic control, body weight, and lipid profile for type 2 diabetes: systematic review and network meta-analysis. *BMJ* 2024;**384**:e076410.
71. Patel S, Niazi SK. Emerging frontiers in GLP-1 therapeutics: a comprehensive evidence base. *Pharmaceutics* 2025;**17**:1036.
72. Iepsen EW, Zhang J, Thomsen HS, Hansen EL, Hollensted M, Madsbad S, et al. Patients with obesity caused by melanocortin-4 receptor mutations can be treated with a glucagon-like peptide-1 receptor agonist. *Cell Metab* 2018;**28**:23−32.e3.
73. Bhatnagar P, Ahmad NN, Li X, Coghlan M, Kaplan LM, Farooqi IS. Tirzepatide leads to weight reduction in people with obesity due to MC4R deficiency. *Nat Med* 2025;**31**:3294−6.
74. Ashlaw EF, Elfers CT, Chichura KS, Miranda IC, McGivney A, Chepurny OG, et al. A melanocortin 4- and glucagon-like peptide 1 receptor multiple agonist for the treatment of diabetes and obesity. *Metabolism* 2026;**174**:156414.
75. Iepsen EW, Have CT, Veedfald S, Madsbad S, Holst JJ, Grarup N, et al. GLP-1 receptor agonist treatment in morbid obesity and type 2 diabetes due to pathogenic homozygous melanocortin-4 receptor mutation: a case report. *Cell Rep Med* 2020;**1**:100006.
76. Amorim MR, Williams NR, Aung O, Ruiz MA, Anokye-Danso F, de Deus JL, et al. Targeting melanocortin 4 receptor to treat sleep-disordered breathing in mice. *J Clin Investig* 2025;**135**:e177823.
77. Oya M, Miyasaka Y, Nakamura Y, Tanaka M, Suganami T, Mashimo T, et al. Age-related ciliopathy: obesogenic shortening of melanocortin-4 receptor-bearing neuronal primary cilia. *Cell Metab* 2024;**36**:1044−58.e10.
78. Wang Y, Bernard A, Comblain F, Yue X, Paillart C, Zhang S, et al. Melanocortin 4 receptor signals at the neuronal primary cilium to control food intake and body weight. *J Clin Investig* 2021;**131**:e142064.
79. Sayk F, Heutling D, Dodt C, Iwen KA, Wellhoner JP, Scherag S, et al. Sympathetic function in human carriers of melanocortin-4 receptor gene mutations. *J Clin Endocrinol Metab* 2010;**95**:1998−2002.
80. Anderson EJP, Ghamari-Langroudi M, Cakir I, Litt MJ, Chen V, Reggiardo RE, et al. Late onset obesity in mice with targeted deletion of potassium inward rectifier Kir7.1 from cells expressing the melanocortin-4 receptor. *J Neuroendocrinol* 2019;**31**:e12670.
81. Ghamari-Langroudi M, Digby GJ, Sebag JA, Millhauser GL, Palomino R, Matthews R, et al. G-protein-independent coupling of MC4R to Kir7.1 in hypothalamic neurons. *Nature* 2015;**520**:94−8.
82. Sharma S, Garfield AS, Shah B, Kleyn P, Ichetovkin I, Moeller IH, et al. Current mechanistic and pharmacodynamic understanding of melanocortin-4 receptor activation. *Molecules* 2019;**24**:1892.
83. Xiang Z, Litherland SA, Sorensen NB, Proneth B, Wood MS, Shaw AM, et al. Pharmacological characterization of 40 human melanocortin-4 receptor polymorphisms with the endogenous proopiomelanocortin-derived agonists and the agouti-related protein (AGRP) antagonist. *Biochemistry* 2006;**45**:7277−88.
84. Matsumura S, Miyakita M, Miyamori H, Kyo S, Shima D, Yokokawa T, et al. Stimulation of G_s signaling in MC4R cells by DREADD increases energy expenditure, suppresses food intake, and increases locomotor activity in mice. *Am J Physiol Endocrinol Metab* 2022;**322**:E436−45.
85. Khong K, Kurtz SE, Sykes RL, Cone RD. Expression of functional melanocortin-4 receptor in the hypothalamic GT1-1 cell line. *Neuroendocrinology* 2001;**74**:193−201.

86. Yang L. Neuronal cAMP/PKA signaling and energy homeostasis. *Adv Exp Med Biol* 2018;**1090**:31−48.
87. Ju SH, Yun H, Oh Y, Choi Y, Sohn JW. Melanocortin-4 receptors activate sympathetic preganglionic neurons and elevate blood pressure *via* TRPV1. *Cell Rep* 2022;**41**:111579.
88. Sohn JW, Harris LE, Berglund ED, Liu T, Vong L, Lowell BB, et al. Melanocortin 4 receptors reciprocally regulate sympathetic and parasympathetic preganglionic neurons. *Cell* 2013;**152**:612−9.
89. Pauza AG, Thakkar P, Shen X, Felippe ISA, Roßmann K, Oya M, et al. Melanocortin system activates carotid body arterial chemoreceptors in hypertension. *Circ Res* 2025;**137**:967−82.
90. Chen M, Berger A, Kablan A, Zhang J, Gavrilova O, Weinstein LS. Gsα deficiency in the paraventricular nucleus of the hypothalamus partially contributes to obesity associated with Gsα mutations. *Endocrinology* 2012;**153**:4256−65.
91. Newman EA, Chai BX, Zhang W, Li JY, Ammori JB, Mulholland MW. Activation of the melanocortin-4 receptor mobilizes intracellular free calcium in immortalized hypothalamic neurons. *J Surg Res* 2006;**132**:201−7.
92. Ballesteros JA, Weinstein H. Integrated methods for the construction of three-dimensional models and computational probing of structure-function relations in G protein-coupled receptors. *Methods Neurosci* 1995;**25**:366−428.
93. Kang C. Setmelanotide in obesity: a profile of its use. *Drugs Ther Perspect* 2022;**38**:308−15.
94. Hammad MM, Mohammad A, Alam-Eldin N, Madhu D, Al-Mulla F, Abu-Farha M, et al. Structural analysis of setmelanotide binding to MC4R variants in comparison to wild-type receptor. *Life Sci* 2022; **307**:120857.
95. Koerperich ZM, Ericson MD, Freeman KT, Speth RC, Pogozheva ID, Mosberg HI, et al. Incorporation of agouti-related protein (AgRP) human single nucleotide polymorphisms (SNPs) in the AgRP-derived macrocyclic scaffold c[Pro-Arg-Phe-Phe-Asn-Ala-Phe-dPro] decreases melanocortin-4 receptor antagonist potency and results in the discovery of melanocortin-5 receptor antagonists. *J Med Chem* 2020; **63**:2194−208.
96. McNulty JC, Thompson DA, Bolin KA, Wilken J, Barsh GS, Millhauser GL. High-resolution NMR structure of the chemically-synthesized melanocortin receptor binding domain AGRP (87−132) of the agouti-related protein. *Biochemistry* 2001;**40**:15520−7.
97. Tao YX. Constitutive activity in melanocortin-4 receptor: biased signaling of inverse agonists. *Adv Pharmacol* 2014;**70**:135−54.
98. Chai BX, Neubig RR, Millhauser GL, Thompson DA, Jackson PJ, Barsh GS, et al. Inverse agonist activity of agouti and agouti-related protein. *Peptides* 2003;**24**:603−9.
99. Adan RA, Kas MJ. Inverse agonism gains weight. *Trends Pharmacol Sci* 2003;**24**:315−21.
100. Haskell-Luevano C, Monck EK. Agouti-related protein functions as an inverse agonist at a constitutively active brain melanocortin-4 receptor. *Regul Pept* 2001;**99**:1−7.
101. Brouwers B, de Oliveira EM, Marti-Solano M, Monteiro FBF, Laurin S-A, Keogh JM, et al. Human MC4R variants affect endocytosis, trafficking and dimerization revealing multiple cellular mechanisms involved in weight regulation. *Cell Rep* 2021;**34**: 108862.
102. Rashid M, Gavrilova O, Cui Z, Wess J. 98-OR: Melanocortin 4 receptor-mediated effects require beta-arrestin 2 signaling. *Diabetes* 2025;**74**:98.
103. Ojeda-Naharros I, Das T, Castro RA, Bazan JF, Vaisse C, Nachury MV. Tonic ubiquitination of the central body weight regulator melanocortin receptor 4 (MC4R) promotes its constitutive exit from cilia. *PLoS Biol* 2025;**23**:e3003025.
104. Hernandez CC, Gimenez LE, Cone RD. Automated patch clamp recordings of GPCR-gated ion channels: targeting the MC4-R/Kir7.1 potassium channel complex. *Methods Mol Biol* 2024;**2796**:229−48.
105. Litt MJ, Cone RD, Ghamari-Langroudi M. Characterization of MC4R regulation of the Kir7.1 channel using the Tl^+ flux assay. *Methods Mol Biol* 2018;**1684**:211−22.
106. Hernandez CC, Gimenez LE, Dahir NS, Peisley A, Cone RD. The unique structural characteristics of the Kir 7.1 inward rectifier potassium channel: a novel player in energy homeostasis control. *Am J Physiol Cell Physiol* 2023;**324**:C694−706.
107. Peisley A, Hernandez CC, Dahir NS, Koepping L, Raczkowski A, Su M, et al. Structure of the ion channel Kir7.1 and implications for its function in normal and pathophysiologic states. *bioRxiv* 2024. Available from: https://doi.org/10.1101/2024.06.07.597981.
108. Haddad HK, Mercado-Reyes JI, Mustafá ER, D'Souza SP, Chung CS, Nestor RRM, et al. Hypothalamic opsin 3 suppresses MC4R signaling and potentiates Kir7.1 to promote food consumption. *Proc Natl Acad Sci U S A* 2025;**122**:e2403891122.
109. Rodríguez Rondón AV, Welling MS, van den Akker ELT, van Rossum EFC, Boon EMJ, van Haelst MM, et al. MC4R variants modulate α-MSH and setmelanotide induced cellular signaling at multiple levels. *J Clin Endocrinol Metab* 2024;**109**:2452−66.
110. Chen M, Celik A, Georgeson KE, Harmon CM, Yang Y. Molecular basis of melanocortin-4 receptor for AGRP inverse agonism. *Regul Pept* 2006;**136**:40−9.
111. Feng W, Zhou Q, Chen X, Dai A, Cai X, Liu X, et al. Structural insights into ligand recognition and subtype selectivity of the human melanocortin-3 and melanocortin-5 receptors. *Cell Discov* 2023;**9**:81.
112. Yin YL, Ye C, Zhou F, Wang J, Yang D, Yin W, et al. Molecular basis for kinin selectivity and activation of the human bradykinin receptors. *Nat Struct Mol Biol* 2021;**28**:755−61.
113. Koehl A, Hu H, Maeda S, Zhang Y, Qu Q, Paggi JM, et al. Structure of the μ-opioid receptor-G_i protein complex. *Nature* 2018;**558**:547−52.
114. Moore MN, Person KL, Robleto VL, Alwin AR, Krusemark CL, Foster N, et al. Designing allosteric modulators to change GPCR G protein subtype selectivity. *Nature* 2025;**648**:229−38.
115. Kobayashi K, Kawakami K, Kusakizako T, Tomita A, Nishimura M, Sawada K, et al. Class B1 GPCR activation by an intracellular agonist. *Nature* 2023;**618**:1085−93.
116. Duan J, Liu H, Zhao F, Yuan Q, Ji Y, Cai X, et al. GPCR activation and GRK2 assembly by a biased intracellular agonist. *Nature* 2023; **620**:676−81.
117. Slosky LM, Bai Y, Toth K, Ray C, Rochelle LK, Badea A, et al. β-Arrestin-biased allosteric modulator of NTSR1 selectively attenuates addictive behaviors. *Cell* 2020;**181**:1364-79.e14.
118. Clement K, Biebermann H, Farooqi IS, Van der Ploeg L, Wolters B, Poitou C, et al. MC4R agonism promotes durable weight loss in patients with leptin receptor deficiency. *Nat Med* 2018;**24**:551−5.
119. Ericson MD, Lensing CJ, Fleming KA, Schlasner KN, Doering SR, Haskell-Luevano C. Bench-top to clinical therapies: a review of melanocortin ligands from 1954 to 2016. *Biochim Biophys Acta Mol Basis Dis* 2017;**1863**:2414−35.
120. Oosterom J, Nijenhuis WA, Schaaper WM, Slootstra J, Meloen RH, Gispen WH, et al. Conformation of the core sequence in melanocortin peptides directs selectivity for the melanocortin MC3 and MC4 receptors. *J Biol Chem* 1999;**274**:16853−60.
121. Abdel-Malek ZA. Melanocortin receptors: their functions and regulation by physiological agonists and antagonists. *Cell Mol Life Sci* 2001;**58**:434−41.
122. Daoussis D, Antonopoulos I, Yiannopoulos G, Andonopoulos AP. ACTH as first line treatment for acute gout in 181 hospitalized patients. *Jt Bone Spine* 2013;**80**:291−4.
123. Luger TA, Böhm M. An α-MSH analog in erythropoietic protoporphyria. *J Invest Dermatol* 2015;**135**:929−31.
124. Wensink D, Wagenmakers M, Langendonk JG. Afamelanotide for prevention of phototoxicity in erythropoietic protoporphyria. *Expert Rev Clin Pharmacol* 2021;**14**:151−60.
125. Edinoff AN, Sanders NM, Lewis KB, Apgar TL, Cornett EM, Kaye AM, et al. Bremelanotide for treatment of female hypoactive sexual desire. *Neurol Int* 2022;**14**:75−88.
126. Kingsberg SA, Clayton AH, Portman D, Williams LA, Krop J, Jordan R, et al. Bremelanotide for the treatment of hypoactive sexual desire disorder: two randomized phase 3 trials. *Obstet Gynecol* 2019; **134**:899−908.

127. Krishna R, Gumbiner B, Stevens C, Musser B, Mallick M, Suryawanshi S, et al. Potent and selective agonism of the melanocortin receptor 4 with MK-0493 does not induce weight loss in obese human subjects: energy intake predicts lack of weight loss efficacy. *Clin Pharmacol Ther* 2009;**86**:659−66.
128. ClinicalTrials.gov. An open-label extension study of bivamelagon in participants with acquired hypothalamic obesity. *Identifier*, **NCT07156578**. Available from: https://clinicaltrials.gov/study/NCT07156578.
129. Royalty JE, Konradsen G, Eskerod O, Wulff BS, Hansen BS. Investigation of safety, tolerability, pharmacokinetics, and pharmacodynamics of single and multiple doses of a long-acting α-MSH analog in healthy overweight and obese subjects. *J Clin Pharmacol* 2014;**54**:394−404.
130. Skowronski AA, Morabito MV, Mueller BR, Lee S, Hjorth S, Lehmann A, et al. Effects of a novel MC4R agonist on maintenance of reduced body weight in diet-induced obese mice. *Obesity* 2014;**22**:1287−95.
131. ClinicalTrials.gov. A phase 1 study of RM-718 in participants with obesity or MC4R pathway diseases. *Identifier*, **NCT06239116**. Available from: https://clinicaltrials.gov/study/NCT06239116.
132. Garnsey MR, Smith AC, Polivkova J, Arons AL, Bai G, Blakemore C, et al. Discovery of the potent and selective MC4R antagonist PF-07258669 for the potential treatment of appetite loss. *J Med Chem* 2023;**66**:3195−211.
133. Zhu X, Potterfield R, Gruber KA, Zhang E, Newton SD, Norgard MA, et al. Melanocortin-4 receptor antagonist TCMCB07 alleviates chemotherapy-induced anorexia and weight loss in rats. *J Clin Investig* 2024;**135**:e181305.
134. Al-Obeidi F, Castrucci AM, Hadley ME, Hruby VJ. Potent and prolonged acting cyclic lactam analogues of α-melanotropin: design based on molecular dynamics. *J Med Chem* 1989;**32**:2555−61.
135. Chen S, Zhao L, Sherchan P, Ding Y, Yu J, Nowrangi D, et al. Activation of melanocortin receptor 4 with RO27-3225 attenuates neuroinflammation through AMPK/JNK/p38 MAPK pathway after intracerebral hemorrhage in mice. *J Neuroinflammation* 2018;**15**:106.
136. Lansdell MI, Hepworth D, Calabrese A, Brown AD, Blagg J, Burring DJ, et al. Discovery of a selective small-molecule melanocortin-4 receptor agonist with efficacy in a pilot study of sexual dysfunction in humans. *J Med Chem* 2010;**53**:3183−97.
137. Sebhat IK, Martin WJ, Ye Z, Barakat K, Mosley RT, Johnston DB, et al. Design and pharmacology of *N*-[(3*R*)-1,2,3,4-tetrahydroisoquinolinium-3-ylcarbonyl]-(1*R*)-1-(4-chlorobenzyl)-2-[4-cyclohexyl-4-(1*H*-1,2,4-triazol-1-ylmethyl)piperidin-1-yl]-2-oxoethylamine (1), a potent, selective, melanocortin subtype-4 receptor agonist. *J Med Chem* 2002;**45**:4589−93.
138. Hruby VJ, Lu D, Sharma SD, Castrucci AL, Kesterson RA, al-Obeidi FA, et al. Cyclic lactam alpha-melanotropin analogues of Ac-Nle4-cyclo[Asp5, D-Phe7, Lys10] α-melanocyte-stimulating hormone-(4−10)-NH_2 with bulky aromatic amino acids at position 7 show high antagonist potency and selectivity at specific melanocortin receptors. *J Med Chem* 1995;**38**:3454−61.
139. Grieco P, Han G, Weinberg D, Van der Ploeg LH, Hruby VJ. Design and synthesis of highly potent and selective melanotropin analogues of SHU9119 modified at position 6. *Biochem Biophys Res Commun* 2002;**292**:1075−80.
140. Poitout L, Brault V, Sackur C, Bernetière S, Camara J, Plas P, et al. Identification of a novel series of benzimidazoles as potent and selective antagonists of the human melanocortin-4 receptor. *Bioorg Med Chem Lett* 2007;**17**:4464−70.
141. Fleming KA, Ericson MD, Freeman KT, Adank DN, Lunzer MM, Wilber SL, et al. Structure-activity relationship studies of a macrocyclic AGRP-mimetic scaffold c[Pro-Arg-Phe-Phe-Asn-Ala-Phe-DPro] yield potent and selective melanocortin-4 receptor antagonists and melanocortin-5 receptor inverse agonists that increase food intake in mice. *ACS Chem Neurosci* 2018;**9**:1141−51.
142. Goit RK, Ng TC, Tam KC, Tsang JKW, Taylor AW, Lo ACY. Neuropeptide α-melanocyte-stimulating hormone promotes neurological recovery and repairs cerebral ischemia/reperfusion injury in type 1 diabetes. *Neurochem Res* 2022;**47**:394−408.
143. Biolcati G, Marchesini E, Sorge F, Barbieri L, Schneider-Yin X, Minder EI. Long-term observational study of afamelanotide in 115 patients with erythropoietic protoporphyria. *Br J Dermatol* 2015;**172**:1601−12.
144. Sawyer TK, Sanfilippo PJ, Hruby VJ, Engel MH, Heward CB, Burnett JB, et al. 4-Norleucine, 7-D-phenylalanine-α-melanocyte-stimulating hormone: a highly potent α-melanotropin with ultralong biological activity. *Proc Natl Acad Sci U S A* 1980;**77**:5754−8.
145. Grieco P, Balse PM, Weinberg D, MacNeil T, Hruby VJ. D-Amino acid scan of γ-melanocyte-stimulating hormone: importance of Trp(8) on human MC3 receptor selectivity. *J Med Chem* 2000;**43**:4998−5002.
146. Fan W, Boston BA, Kesterson RA, Hruby VJ, Cone RD. Role of melanocortinergic neurons in feeding and the agouti obesity syndrome. *Nature* 1997;**385**:165−8.
147. Irani BG, Xiang Z, Yarandi HN, Holder JR, Moore MC, Bauzo RM, et al. Implication of the melanocortin-3 receptor in the regulation of food intake. *Eur J Pharmacol* 2011;**660**:80−7.
148. Vink T, Hinney A, van Elburg AA, van Goozen SH, Sandkuijl LA, Sinke RJ, et al. Association between an agouti-related protein gene polymorphism and anorexia nervosa. *Mol Psychiatry* 2001;**6**:325−8.
149. McNulty JC, Jackson PJ, Thompson DA, Chai B, Gantz I, Barsh GS, et al. Structures of the agouti signaling protein. *J Mol Biol* 2005;**346**:1059−70.
150. Wan GJ, Niewoehner J, Hayes K. Acthar Gel (RCI): a narrative literature review of clinical and economic evidence. *Clinicoecon Outcomes Res* 2023;**15**:499−512.
151. Langendonk JG, Balwani M, Anderson KE, Bonkovsky HL, Anstey AV, Bissell DM, et al. Afamelanotide for erythropoietic protoporphyria. *N Engl J Med* 2015;**373**:48−59.
152. Castejón-Griñán M, Herraiz C, Olivares C, Jiménez-Cervantes C, García-Borrón JC. cAMP-independent non-pigmentary actions of variant melanocortin 1 receptor: AKT-mediated activation of protective responses to oxidative DNA damage. *Oncogene* 2018;**37**:3631−46.
153. Chen S, Zhu B, Yin C, Liu W, Han C, Chen B, et al. Palmitoylation-dependent activation of MC1R prevents melanomagenesis. *Nature* 2017;**549**:399−403.
154. Durek T, Cromm PM, White AM, Schroeder CI, Kaas Q, Weidmann J, et al. Development of novel melanocortin receptor agonists based on the cyclic peptide framework of sunflower trypsin inhibitor-1. *J Med Chem* 2018;**61**:3674−84.
155. Kühnen P, Clément K, Wiegand S, Blankenstein O, Gottesdiener K, Martini LL, et al. Proopiomelanocortin deficiency treated with a melanocortin-4 receptor agonist. *N Engl J Med* 2016;**375**:240−6.
156. Kleinau G, Heyder NA, Tao YX, Scheerer P. Structural complexity and plasticity of signaling regulation at the melanocortin-4 receptor. *Int J Mol Sci* 2020;**21**:5728.
157. Kanti V, Puder L, Jahnke I, Krabusch PM, Kottner J, Vogt A, et al. A melanocortin-4 receptor agonist induces skin and hair pigmentation in patients with monogenic mutations in the leptin-melanocortin pathway. *Skin Pharmacol Physiol* 2021;**34**:307−16.
158. Yang Y, Cai M, Chen M, Qu H, McPherson D, Hruby V, et al. Key amino acid residues in the melanocortin-4 receptor for nonpeptide THIQ specific binding and signaling. *Regul Pept* 2009;**155**:46−54.
159. Martin WJ, McGowan E, Cashen DE, Gantert LT, Drisko JE, Hom GJ, et al. Activation of melanocortin MC4 receptors increases erectile activity in rats ex copula. *Eur J Pharmacol* 2002;**454**:71−9.
160. Van der Ploeg LH, Martin WJ, Howard AD, Nargund RP, Austin CP, Guan X, et al. A role for the melanocortin 4 receptor in sexual function. *Proc Natl Acad Sci U S A* 2002;**99**:11381−6.
161. Hadley ME. Discovery that a melanocortin regulates sexual functions in male and female humans. *Peptides* 2005;**26**:1687−9.
162. Tao YX. The melanocortin-4 receptor: physiology, pharmacology, and pathophysiology. *Endocr Rev* 2010;**31**:506−43.

163. Mo XL, Tao YX. Activation of MAPK by inverse agonists in six naturally occurring constitutively active mutant human melanocortin-4 receptors. *Biochim Biophys Acta* 2013;**1832**:1939−48.
164. Huang H, Wang W, Tao YX. Pharmacological chaperones for the misfolded melanocortin-4 receptor associated with human obesity. *Biochim Biophys Acta Mol Basis Dis* 2017;**1863**:2496−507.
165. Hong Q, Bakshi RK, Palucki BL, Park MK, Ye Z, He S, et al. Discovery of a piperazine urea based compound as a potent, selective, orally bioavailable melanocortin subtype-4 receptor partial agonist. *Bioorg Med Chem Lett* 2011;**21**:2330−4.
166. Drabkin M, Birk OS, Birk R. Heterozygous *versus* homozygous phenotype caused by the same MC4R mutation: novel mutation affecting a large consanguineous kindred. *BMC Med Genet* 2018;**19**: 135.
167. Harno E, Gali Ramamoorthy T, Coll AP, White A. POMC: the physiological power of hormone processing. *Physiol Rev* 2018;**98**: 2381−430.
168. Kusminski CM, Perez-Tilve D, Müller TD, DiMarchi RD, Tschöp MH, Scherer PE. Transforming obesity: the advancement of multi-receptor drugs. *Cell* 2024;**187**:3829−53.
169. Secher A, Jelsing J, Baquero AF, Hecksher-Sørensen J, Cowley MA, Dalbøge LS, et al. The arcuate nucleus mediates GLP-1 receptor agonist liraglutide-dependent weight loss. *J Clin Investig* 2014;**124**: 4473−88.
170. Fortin SM, Chen J, Hayes MR. Hindbrain melanocortin 3/4 receptors modulate the food intake and body weight suppressive effects of the GLP-1 receptor agonist, liraglutide. *Physiol Behav* 2020;**220**: 112870.
171. Panaro BL, Tough IR, Engelstoft MS, Matthews RT, Digby GJ, Moller CL, et al. The melanocortin-4 receptor is expressed in enteroendocrine L cells and regulates the release of peptide YY and glucagon-like peptide 1 *in vivo*. *Cell Metab* 2014;**20**:1018−29.
172. Sun EW, Iepsen EW, Pezos N, Lumsden AL, Martin AM, Schober G, et al. A gut-intrinsic melanocortin signaling complex augments L-cell secretion in humans. *Gastroenterology* 2021;**161**:536-47.e2.
173. A Phase 2 study evaluating the co-administration of bremelanotide with tirzepatide for the treatment of obesity (BMT-801). Identifier: NCT06565611. Available from: https://clinicaltrials.gov/study/NCT06565611.
174. Ayers KL, Glicksberg BS, Garfield AS, Longerich S, White JA, Yang P, et al. Melanocortin 4 receptor pathway dysfunction in obesity: patient stratification aimed at MC4R agonist treatment. *J Clin Endocrinol Metab* 2018;**103**:2601−12.
175. He Y, Brouwers B, Liu H, Liu H, Lawler K, Mendes de Oliveira E, et al. Human loss-of-function variants in the serotonin 2C receptor associated with obesity and maladaptive behavior. *Nat Med* 2022;**28**: 2537−46.
176. Yi CX, Kalsbeek A, Tschöp MH. Autonomic MC sets the metabolic tone. *Cell Metab* 2011;**13**:121−3.
177. Singh U, Saito K, Cui H. The role of PVN MC4R neural pathway in autonomic control of metabolic homeostasis and cardiovascular function. *FASEB J* 2020;**34**:1.
178. DiFeliceantonio AG, Kenny PJ. Melanocortin 4 receptors switch reward to aversion. *J Clin Investig* 2018;**128**:2757−9.
179. Ryan KK, Mul JD, Clemmensen C, Egan AE, Begg DP, Halcomb K, et al. Loss of melanocortin-4 receptor function attenuates HPA responses to psychological stress. *Psychoneuroendocrinology* 2014;**42**: 98−105.
180. van Santen HM, Denzer C, Müller HL. Could setmelanotide be the game-changer for acquired hypothalamic obesity?. *Front Endocrinol* 2024;**14**:1307889.
181. Haqq AM, Chung WK, Dollfus H, Haws RM, Martos-Moreno GÁ, Poitou C, et al. Efficacy and safety of setmelanotide, a melanocortin-4 receptor agonist, in patients with Bardet-Biedl syndrome and Alström syndrome: a multicentre, randomised, double-blind, placebo-controlled, phase 3 trial with an open-label period. *Lancet Diabetes Endocrinol* 2022;**10**:859−68.
182. Zhu X, Callahan MF, Gruber KA, Szumowski M, Marks DL. Melanocortin-4 receptor antagonist TCMCB07 ameliorates cancer- and chronic kidney disease−associated cachexia. *J Clin Investig* 2020; **130**:4921−34.
183. Akinci B, Meral R, Rus D, Hench R, Neidert AH, DiPaola F, et al. The complicated clinical course in a case of atypical lipodystrophy after development of neutralizing antibody to metreleptin: treatment with setmelanotide. *Endocrinol Diabetes Metab Case Rep* 2020; **2020**:19−139.
184. da Silva AA, do Carmo JM, Wang Z, Hall JE. Melanocortin-4 receptors and sympathetic nervous system activation in hypertension. *Curr Hypertens Rep* 2019;**21**:46.
185. Reininghaus N, Paisdzior S, Höpfner F, Jyrch S, Cetindag C, Scheerer P, et al. A setmelanotide-like effect at MC4R is achieved by MC4R dimer separation. *Biomolecules* 2022;**12**:1119.
186. Rediger A, Piechowski CL, Habegger K, Grüters A, Krude H, Tschöp MH, et al. MC4R dimerization in the paraventricular nucleus and GHSR/MC3R heterodimerization in the arcuate nucleus: is there relevance for body weight regulation?. *Neuroendocrinology* 2012;**95**: 277−88.
187. Sohail I, Laurin SA, Kleinau G, Chunilal V, Morton A, Brenlla A, et al. MRAP2 modifies the signaling and oligomerization state of the melanocortin-4 receptor. *Nat Commun* 2025;**16**:8324.
188. Bernard A, Ojeda Naharros I, Yue X, Mifsud F, Blake A, Bourgain-Guglielmetti F, et al. MRAP2 regulates energy homeostasis by promoting primary cilia localization of MC4R. *JCI Insight* 2023;**8**: e155900.
189. Wen ZY, Liu T, Qin CJ, Zou YC, Wang J, Li R, et al. MRAP2 interaction with melanocortin-4 receptor in snakeHead (*Channa argus*). *Biomolecules* 2021;**11**:481.
190. Rao YZ, Chen R, Zhang Y, Tao YX. Orange-spotted grouper melanocortin-4 receptor: modulation of signaling by MRAP2. *Gen Comp Endocrinol* 2019;**284**:113234.
191. Rouault AAJ, Srinivasan DK, Yin TC, Lee AA, Sebag JA. Melanocortin receptor accessory proteins (MRAPs): functions in the melanocortin system and beyond. *Biochim Biophys Acta Mol Basis Dis* 2017;**1863**:2462−7.
192. Kay EI, Botha R, Montgomery JM, Mountjoy KG. hMRAPα, but not hMRAP2, enhances hMC4R constitutive activity in HEK293 cells and this is not dependent on hMRAPα induced changes in hMC4R complex *N*-linked glycosylation. *PLoS One* 2015;**10**:e0140320.