Published in final edited form as:
Cell Mol Life Sci. 2008 September ; 65(17): 2721–2731. doi:10.1007/s00018-008-8104-4.

The Role of the Agouti-Related Protein in Energy Balance Regulation

O. Ilnytska and **G. Argyropoulos***
Pennington Biomedical Research Center, LSU System, Baton Rouge, Louisiana 70809 (USA)

Abstract

The Agouti-Related Protein (AgRP) is a powerful orexigenic peptide that increases food intake when ubiquitously overexpressed or when administered centrally. AgRP-deficiency, on the other hand, leads to increased metabolic rate and a longer lifespan when mice consume a high fat diet. In humans, AgRP polymorphisms have been consistently associated with resistance to fatness in Blacks and Whites and resistance to the development of type-2 diabetes in African Blacks. Systemically administered AgRP accumulates in the liver, the adrenal gland and fat tissue while recent findings suggest that AgRP may also have inverse agonist effects, both centrally and peripherally. AgRP could thus modulate energy balance via different actions. Its absence or reduced functionality may offer a benefit both in terms of bringing about negative energy balance in obesigenic environments, as well as leading to an increased lifespan.

Keywords

Hypothalamus; adrenal gland; longevity; polymorphism

Introduction

Since its discovery in 1997 [1,2], the Agouti-Related Protein (AgRP) has come a long way in being considered an important modulator of energy balance [3]. In 1997, Ollmann and co-workers [1], in parallel with Shutter and coworkers [2], identified a hypothalamic protein with high sequence homology to Agouti and termed it Agouti-Related Transcript and subsequently Agouti-Related Protein (AgRP). AgRP was suggested to play a significant role in the regulation of energy homeostasis because of its pattern of expression and physiological effects [4-7]. Indeed, hypothalamic AgRP is elevated in obese and diabetic mice [2] while transgenic mice overexpressing AgRP ubiquitously are hyperphagic and obese [8]. The main mode of AgRP action involves its antagonistic binding to melanocortin receptors 3 and 4 (MC3R & MC4R) which are normally targeted by alpha Melanocyte Stimulating Hormone (αMSH). Later studies (discussed in detail later) have shown that neurons in the hypothalamus that express AgRP are essential for controlling energy homeostasis [9-11]. AgRP is therefore a significant modulator of energy balance and has been considered a candidate gene for human obesity. The present review describes recent advances in our understanding of AgRP action in mouse models and humans.

The human AgRP gene is a relatively short gene spanning 1.1kb on chromosome 16q22. It consists of 4 exons (one 5′ non-coding and three coding) and encodes a 132 amino acid (aa)

*Corresponding author. Fax: (225) 763-3030, argyrog@pbrc.edu

protein, with the rodent ortholog encoding a 131 aa protein [2,12,13]. The AgRP protein contains 9 cysteine residues at conserved positions that form disulfide bridges and are important for its function [2]. Human and murine AgRP expression profiles exhibit a predominant transcript in the hypothalamus, the subthalamic nucleus, and a shorter transcript lacking the 5" non-coding exon in the adrenal gland, testis, lung, and dorsal root ganglia [2,14]. Although at present it is unclear whether these transcripts arise from differential splicing or from two independently regulated promoters, the 5′ untranslated exon has significant promoter activity in periphery-derived cell lines [13]. This finding suggests that this exon may play a role in the peripheral expression of AgRP, and supports the existence of two active promoters, each responsible for either brain or periphery-specific (the adrenal gland, testis, and lung) expression [13]. AgRP orthologs have also been found in pig [15], sheep [16], fugu fish [17], zebrafish [18], goldfish [19], Japanese quail [20,21] and ring doves [22,23]. In many of these species, AgRP levels are upregulated by fasting [16,24], which is suggestive of a conserved role for AgRP in energy homeostasis.

Physiological properties of AgRP

Most studies focusing on AgRP function have used carboxyl-terminus peptides that mimic the effect of the full-length protein. This approach appears legitimate, as synthetic peptides of the amino-terminus, AgRP(aa25-51), and the mid-portion, AgRP(aa54-82), of the human AgRP protein were devoid of antagonistic action for α-MSH [25,26], whereas a synthetic variant containing the 46 C-terminal residues, AgRP(aa87-132), was active and equipotent to the mature mouse homolog [27-30]. Moreover, the AgRP(aa87-132) peptide was able to bind antagonistically to the melanocortin receptors MC3R, MC4R, and MC5R, thus inhibiting binding of α-MSH [31-33].

NMR structure analysis of this AgRP domain (aa87-132) revealed an inhibitor cysteine-knot structure [34] which makes contact with melanocortin receptors 3 and 4 with two loops present in this structure [35]. Recently, elongation of the minimum core decapeptide Yc[CRFFNAFC]Y of AgRP, which acts as an antagonist at the MC4R but not the MC3R, by two amino acids at either the C- or the N-terminus conferred antagonistic function also on the MC3R, resembling the profile of full length AgRP [36]. The utility of these peptides is underscored by the fact that recent studies have shown that physiological AgRP undergoes post-translational cleavage by proprotein convertase 1 to generate the carboxyl-terminus $^{[83\text{-}132]}$AgRP peptide [37] and amino-terminus $^{[25\text{-}51]}$AgRP, and mid-section $^{[54\text{-}82]}$AgRP peptides [38].

In addition to the C-terminus, the N-terminal parts of AgRP, which are unable to bind to melanocortin receptors, could also have significant effects on energy balance regulation [39]. A single, i.c.v. injection of AgRP (aa83-132) increased cumulative food intake, whereas N-terminal parts AgRP (aa25-51) and AgRP (aa54-82) did not affect the amount of food consumption. However, injection of any of the three portions of AgRP resulted in decreased oxygen consumption and colonic temperature, both of which are readouts for energy expenditure, while the two non-C-terminus parts of AgRP increased body weight and epididymal/mesenteric fat weight, despite the absence of hyperphagia and cross-reactivity with MC4R [39]. Additional experiments are necessary to confirm and elucidate these interesting data. One possible explanation for this melanocortin independent effect is a potential interaction with co-receptors. One such example could be syndecan-3 [40,41], which can only bind to N-terminal AgRP. A repeat of the study, e.g. in syndecan-3 knockout mice or by altering the availability of membrane-bound syndecan-3, together with further mutagenesis and *in vitro* binding experiments would be helpful to understand further the function of all AgRP protein concatamers.

Multiple hormonal signals influence AgRP expression. Long acting satiety signals such as leptin or insulin act to decrease AgRP, while maintaining physiological levels of these hormones blocks fasting-induced increases of AgRP [42-44]. Loss of leptin or insulin receptors within the brain leads to increased AgRP expression, while AgRP can also be upregulated in leptin-deficient (ob/ob) obese mice irrespective of fasting [1,2]. These observations suggest that these hormones reduce appetite in part by inhibiting AgRP expression [2,45]. Recent work indicates that these hormones can acutely alter membrane potential and reduce neuronal firing from neurons containing NPY, suggesting that leptin and insulin rapidly inhibit the activity of AgRP/NPY neurons [46]. In leptin deficient mice, however, leptin plays a neurotrophic role during neonatal development of the hypothalamus by promoting neurite outgrowth from arcuate nucleus neurons in vitro [47]. Although progress has been made in identifying factors downstream of leptin signaling on AgRP [42-44], it remains unclear which exact mechanism leptin uses to regulate AgRP. Potential pathways include AMP-kinase [48,49], PI3K [50,51], and the JAK-STAT [52-55] pathway but perhaps in a STAT3-independent fashion [56].

The gut-derived protein, ghrelin, has also been implicated in the regulation of AgRP neurons. Unlike leptin and insulin, ghrelin principally acts to stimulate feeding and body weight gain [57,58] by activating NPY/AgRP neurons. Ghrelin is an endogenous ligand for the Growth Hormone Secretagogue Receptor (GHS-R) and has been shown to up-regulate expression of AgRP [59-63]. In addition, administration of ghrelin acutely induces c-Fos (a marker of neuronal activation) within neurons containing NPY (and presumably AgRP). Genetic evidence also supports a critical role for the NPY/AgRP neurons in mediating ghrelin's action, since AgRP/NPY double knockout mice are resistant to ghrelin-dependant increase of food intake [64]. However, mice lacking only AgRP or only NPY do not display this phenotype, whereas AgRP expression was shown by another group to be normal in ghrelin-deficient mice [65]. These findings suggest that ghrelin may not be required for the upregulation of AgRP but, when ghrelin is administered exogenously or when the gene is upregulated it may also lead to upregulation of AgRP and enhanced food intake.

In addition to leptin, insulin, and ghrelin, glucocorticoids have been implicated in the regulation of energy homeostasis and removal of glucocorticoid signaling (for instance, by adrenalectomy) ameliorates obesity in a number of physiological and genetic models. Adrenalectomy decreases sensitivity to both AgRP [66] and NPY [67] while increasing the sensitivity to α-MSH [66] and leptin [66,68]. In a different study, adrenalectomy blocked fasting-induced increases in AgRP [69]. Exogenous administration of glucocorticoids, on the other hand, increased food intake, body weight, as well as AgRP and NPY expression [70]. Another study supportive of a role by glucocorticoids on AgRP expression demonstrated that corticosterone secretion temporally coincided with the rising phase of diurnal AgRP expression [69]. Depletion of corticosterone by adrenalectomy abolished this AgRP diurnal rhythm, which was restored by exogenous corticosterone replacement, highlighting its requirement to maintain the normal diurnal AgRP expression cycle [69]. Together, these observations suggest that glucocorticoids have significant effects on energy homeostasis potentially mediated by action on hypothalamic AgRP/NPY neurons.

In addition to being upregulated by fasting, AgRP is also increased in other physiological situations whereby increased food intake is desirable or necessary. For example, during pregnancy AgRP levels, but not POMC, MC4R or NPY, were elevated in Wistar rats, suggesting that AgRP could play a role in pregnancy-associated hyperphagia [71]. Similarly, AgRP is up-regulated in lactating sheep [72] while ring doves express elevated AgRP levels during the post hatching stages when parents eat more food to feed their young [22]. Some diseases that result in insufficient food intake correlate with reduced levels of AgRP, such as a mouse model of Prader-Willi syndrome in which neonates display failure-to-thrive [73]. In a rat experimental model of anorexia nervosa, central infusion of AgRP prevented self-

starvation by counteracting physical hyperactivity and stimulating food intake [74]. AgRP treatment in tumor-bearing animals led to a maintenance of lean body mass and circadian activity patterns during tumor growth without negatively affecting tumor size [75]. AgRP could therefore modulate disease-related changes in food intake and contribute to maintenance of overall energy balance in response to stress, and hormonal/substrate stimuli.

AgRP also influences physiological systems beyond feeding. In particular, recent evidence suggests that AgRP influences neuroendocrine function by regulating the hypothalamic pituitary axis I.c.v. injection of human AgRP into ovariectomized rhesus monkeys resulted in elevated cortisol, adrenocorticotropin hormone (ACTH), and prolactin (PRL) release [76]. In addition, i.c.v. injected AgRP enhanced the ability of IL-1β to increase ACTH, likely by affecting the function of α-MSH at hypothalamic melanocortin receptors. This observation supports a modulatory role of AgRP in the neuroendocrine responses to inflammation, which itself may possibly promote obesity and type-2 diabetes [77]. Evidence also suggests that AgRP has an inhibitory influence over the hypothalamic-pituitary-thyroid axis, with AgRP administration suppressing hypothalamic Thyroid Stimulating Hormone (TSH)-releasing hormone (TRH) expression and decreasing circulating levels of thyroid hormones [78].

Peripheral actions of AgRP

In addition to the hypothalamic arcuate nucleus, AgRP is robustly expressed in the adrenal gland [79]. Within the adrenal cortex, the short isoform of the rat AgRP is up-regulated during fasting [80], paralleling the increase that occurs within the hypothalamus [24]. Moreover, plasma levels of AgRP are increased in obese men [81] and fasted rats [82]. Nevertheless, the function of AgRP in the periphery remains unresolved. The active isoform of human AgRP (aa83-132) crosses the blood-brain barrier, albeit slowly, [83], raising the possibility for peripherally-expressed AgRP to directly access melanocortin receptors within the brain.

Although only a few studies have focused on peripheral AgRP action, these studies highlight the potential for circulating or locally-produced AgRP to influence peripheral tissues. The obvious question is “what is the role of AgRP in the periphery?”. In all probability, AgRP in the periphery does the same as it does in the hypothalamus: i.e. binds to the melanocortin or perhaps other G protein-coupled receptors. Both AgRP and Agouti have direct effects on adipocytes, influencing the expression of fatty acid synthase and leptin [84-86], and blocking αMSH-dependent effects on leptin gene expression [87]. In addition to effects in adipocytes, AgRP may also have a paracrine role in adrenal gland function, with adrenal-derived AgRP being regulated by glucocorticoids and blocking the induction of corticosterone secretion by α-MSH [88,89]. Furthermore, experiments in the chicken have revealed that AgRP binds to MC3R in the adrenal gland [90,91]. The situation is less clear if a negative feedback loop exists by which AgRP itself acts as a negative regulator of leptin action, as was first suggested by Ebihara et al. [86]. In other experiments, however, central administration of AgRP in rats for three- and seven-day periods resulted in significant increases of plasma leptin levels even when AgRP induced hyperphagia was prevented [92]. In a recent study involving the obesity-susceptible C57BL/6J (C57) and the obesity-resistant CAST/Ei mouse strain, AgRP mRNA was found to be higher in the fasted state in the hypothalamus and the adrenal glands of the leaner CAST/Ei strain instead of in the obesity-susceptible C57 strain. This result has been recapitulated in another obesity-susceptible mouse strain, *tub/tub*, that also had reduced AgRP expression in the hypothalamus [93]. The CAST/E1 strain, however, is known to be hyperactive and to consume higher energy levels per body weight than bigger mice [94-96], thus, AgRP seems to be playing its orexigenic role in keeping these mice in energy balance. These reports present the fresh idea that gene-specific obesity (i.e. the *tub/tub* mutation) or generalized fatness (i.e. the C57 mouse) do not always correlate positively with AgRP levels. It would therefore

appear that AgRP overexpression alone may not lead to increased fatness if a compensatory increase in energy expenditure takes effect.

AgRP also influences neuroendocrine function by regulating the hypothalamic-pituitary axis. Injection of human AgRP i.c.v into ovariectomized rhesus monkeys resulted in elevated cortisol, adrenocorticotropin hormone (ACTH), and prolactin (PRL) release [76]. Evidence also suggests that AgRP has inhibitory effects over the hypothalamic-pituitary-thyroid axis, suppressing hypothalamic Thyroid Stimulating Hormone-Releasing Hormone (TRH) expression and decreasing circulating levels of thyroid hormones [78]. Several studies [29, 33] indicate that AgRP may also act as an inverse agonist again at MC4R. Indeed, it was recently shown that AgRP acts as an inverse agonist in 293 HEK cells by inducing arrestin-mediated endocytosis of MC3R and MC4R [97] but this mechanism requires further experimentation. Furthermore, *in vivo*, a recent study showed that hypothalamic AgRP can modulate energy balance via a mechanism independent of αMSH and MC3&4R or by using a distinct receptor [98]. The latter finding provides an example for a potential action by AgRP that is different from its documented binding to MC3&4R in the arcuate nucleus and raises the possibility for yet-to-be-determined actions, perhaps as an inverse agonist. We would suggest that the generation of transgenic mice overexpressing AgRP specifically in central or peripheral sites (preferably by inducible means) may provide the appropriate tool to study the potential functions of AgRP as an inverse agonist.

Animal models of AgRP

Transgenic mice overexpressing ubiquitously AgRP are hyperphagic, exhibit severe obesity, and have reduced corticosterone levels [8]. In other experiments, RNA interference (siRNA) against AgRP in the ARC resulted in increased metabolic rate and reduced body weight without affecting food intake [99]. In contrast, AgRP driven by mouse beta-actin (pActAgRP) was electroporated into murine leg muscle leading to a significant increase in food intake and body weight that lasted for three weeks after electroporation [100], presenting for the first time an example of the potent effects of peripheral AgRP on energy balance regulation (Table 1). Further experiments along the same design and perhaps targeting other tissues may be performed in the future to study in detail the mode of AgRP's peripheral action when introduced directly into tissues outside of the hypothalamus.

Global AgRP-deficiency, on the other hand, has produced variable phenotypes (Table 1). When first reported, AgRP knockout ($AgRP^{-/-}$) mice displayed normal feeding behavior without changes in body weight and cumulative food intake [101]. In a second report, however, $AgRP^{-/-}$ mice (on a different genetic background) exhibited reduced body weight at 6 months of age which correlated with increased metabolic rate, body temperature, and locomotor activity [102,103].

In a third report, AgRP-deficient mice were unexpectedly found to live significantly longer than their wild type littermates while consuming a high fat diet for the majority of their lives. Specifically, there were no striking metabolic differences between $AgRP^{-/-}$ and the equally obese wild type littermates, but $AgRP^{-/-}$ mice displayed a significantly longer life span [104]. The point estimate of median survival for the $AgRP^{-/-}$ group was 9.8% greater while the significantly low hazard ratio (0.494) suggested that mortality incidence of $AgRP^{-/-}$ mice was less than one-half that of the wild type population. It was concluded that although $AgRP^{-/-}$ mice become morbidly obese consuming a high fat diet (a landmark feature for a shortened life span), they were able to overcome obesity- and age-related patholologies and live significantly longer than their metabolically similar wild type littermates [104]. This finding introduces a novel property for AgRP of potentially conferring a longer lifespan by virtue of its absence. Due to lack of an obvious metabolic phenotype in these long-lived $AgRP^{-/-}$ mice,

further detailed studies would be required to analyze known longevity biomarkers to determine the exact longevity mechanism(s) that AgRP-deficiency recruits to extend lifespan.

In another study, RNA interference (RNAi) in the ARC was used to decrease AgRP expression rather than completely delete the gene, which may be a more physiologically relevant approach. Indeed, RNAi led to a 50% inhibition of AgRP mRNA within the hypothalamus, which was associated with increased metabolic rate and reduced body weight but without changing food intake [99]. This is consistent with conclusions from pair-fed animals treated with AgRP which resulted in increased BAT UCP1 and increased fat mass and leptin levels, potentially regulating overall energy expenditure [105]. Therefore, an approach to moderately decrease AgRP expression levels may provide a means to regulate body weight and increase energy expenditure.

Even though deletion of AgRP in knockout mice did not result in an early and robust phenotype, postnatal neuronal ablation of AgRP did produce more significant outcomes. In an elegant design, transgenic mice were generated using bacterial artificial chromosomes targeting expression of a neurotoxic CAG expanded form of ataxin-3 to reach AgRP-expressing neurons. This approach resulted in 47% loss of AgRP neurons by the age of 16 weeks that significantly reduced body weight [9]. In a parallel study, neuronal ablation of AgRP-expression neurons using diphtheria toxin resulted in a minimal effect when performed in neonates but in significant and rapid starvation in adults [11]. A similar approach has also determined that the appetitive and consummatory aspects of feeding become impaired in a melanocortin-independent manner after AgRP-neuron ablation [106]. These experiments demonstrate the significance of AgRP neurons in the regulation of energy balance in adult life.

Stress conditions and anorexia have been shown to result in a decrease of AgRP levels [74]. The *anx/anx* and Contactin knockout (k.o.) mouse models of anorexia may thus be considered as model organisms to study AgRP expression in pathological conditions associated with loss of appetite. Both of these types of mice display accumulation of AgRP (and NPY) in enlarged arcuate nucleus cell bodies but AgRP is drastically reduced in the nerve fiber network extending from these neurons [107,108], suggesting the presence of a developmental defect. Reports indicate that AgRP is increased in the intact arcuate nucleus in early development but after day 14 post partum, neuronal extensions become significantly defective and the mice exhibit severe anorexia [108,109]. Both *anx/anx* and Contactin k.o. mice are short lived in the homozygous state (3-5 weeks or <3 weeks, respectively) [108] and may present limited opportunities for long-term experimental designs. Yet, their anorectic phenotypes and defective AgRP/NPY system may provide an appropriate paradigm to further our understanding of the central actions of AgRP.

Human genetics of AgRP

In addition to animal models that clearly implicate AgRP in the regulation of energy balance, studies in humans show significant associations of Single Nucleotide Polymorphisms (SNPs) with resistance to the development of obesity in Whites and Blacks, and type-2 diabetes mellitus (T2DM) in Black Africans (Table 2).

Two SNPs have been reported in the 5′-UTR of AgRP (-3019G>A and -38C>T) [110] that were found in Blacks only (Table 1). The *T* allele of the -38C>*T* SNP was associated with leanness in Sierra Leoneans and reduced fatness in the Blacks of the HERITAGE Family Study [111]. In addition, all the diabetics (T2DM) recorded in the Sierra Leonean cohort were CC homozygous (for the -38C>T SNP) suggesting that the *T* allele may predispose to obesity-resistance in Blacks on both sides of the Atlantic, as well as provide a protective mechanism against the development of T2DM in Black Africans.

Two other SNPs have been reported that were not associated with any obese phenotypes [112], but a third SNP, Ala67Thr, was associated with anorexia nervosa [113]. This SNP was found in Whites only [13,114] and a later study showed that heterozygotes were resistant to late-onset obesity [115]. Specifically, the Ala67Thr polymorphism was consistently associated with a reduction of four different measures of human fatness: BMI, fat mass, percent body fat, and abdominal visceral fat, all adjusted for gender and age. Importantly, these findings were true in the case of the parents but not so in the case of the offspring, which suggests that the Ala67Thr genotype could exert its effects in an age-dependent fashion. In other words, the Ala67Thr polymorphism in AgRP could provide a diagnostic marker for preponderance to develop obesity (i.e. the Ala67Ala homozygotes) or resistance against late-onset obesity (i.e. the Ala67Thr heterozygotes) under obesigenic conditions.

In a separate study, the potential association of the two common AgRP SNPs with nutrient selection was examined. In Whites, the Ala67Thr heterozygotes derived a smaller proportion of total energy (E%) from fat than the Ala67Ala homozygotes (Ala67Thr: 29.4% vs Ala67Ala: 31.5%, $p = 0.009$), mainly due to a lower intake of saturated ($p = 0.06$) and monounsaturated fats ($p = 0.01$). Their carbohydrate intake was 2.6 E% units higher compared to the Ala67Ala homozygotes (Ala67Thr: 55.1% vs Ala67Ala: 52.5%, $p = 0.03$). In Blacks, protein intake was associated with the <M-38C>T promoter polymorphism. T/T homozygotes had a significantly lower protein intake than the C-allele carriers (C/C: 16.8%, C/T: 17.2%, T/T: 15.4%, $p = 0.04$). No differences existed between genotypes and total energy or alcohol intakes. These data show that the two ethnic-specific AgRP variants, previously associated with leanness, are also associated with macronutrient intake. This is the first study to report such associations in humans and replication in other populations are needed for confirmation. Animal studies, however, provide support to these findings in humans, whereby i.c.v. injection of AgRP resulted in preference for high fat content diets [116]. Therefore, the Ala67Thr polymorphism could provide a defense against preference for fatty foods.

A rare mutation, +79G>A, was recently identified in the minimal promoter of two White carriers (Table 2). Comparison of the 45-year-old male proband, who was also a carrier of the common Ala67Thr polymorphism, with an age- and weight-matched wild type population showed marginal differences for Resting Metabolic Rate (RMR) and Body Mass Index (BMI). The second carrier, however, was an obese 57-year-old female who was wild type for the Ala67Thr SNP (i.e. Ala67Ala). This individual had reduced RMR relative to the control population [117]. Functional analysis in hypothalamus- and periphery-derived cell lines showed reduced promoter activity for the +79A allele in adrenocortical but not neuronal cell lines, suggesting that it could affect the peripheral expression levels of AgRP. The +79G>A mutation could predispose to body weight gain and reduced RMR (as suggested by the phenotype of the second carrier) while the presence of the Ala67Thr polymorphism could neutralize its effects (as suggested by the phenotype of the proband who was a compound heterozygote for the two mutations).

The plasma levels of AgRP have also been measured in humans. AgRP plasma levels were reported to be elevated in obese men [81], which is consistent with results from some animal models [82], but this finding has been replicated only in lean humans [87,118]. Indeed, a third study showed that AgRP plasma levels were inversely correlated with adiposity and BMI [119]. We have also determined the AgRP plasma levels in African Americans (unpublished data) and found that they also correlated inversely with adiposity. These findings raise doubts about the utility of AgRP plasma levels as a biomarker for assaying the obesity status of humans. Moreover, AgRP plasma levels may reflect the expression of the gene by peripheral sites whose actions remain to be elucidated, and may not represent the expression and action of AgRP in the arcuate nucleus.

In all, polymorphisms in regulatory and coding regions of AgRP could affect energy balance. Most of these genetic variants tend to predispose carriers against body weight gain in an obesigenic environment. The rare +79G>A mutation may be the only exception but additional analysis is required in a population with a higher frequency for this mutation. Since this mutation was found initially in an individual of Mediterranean origin, but not in American Caucasians or African Americans, we suggest that other Mediterranean populations be screened for the presence of this mutation.

Concluding remarks

Chronic overexpression of AgRP leads to hyperphagia and the development of obesity, making AgRP a powerful modulator of energy balance. Emerging experimental evidence also suggests that AgRP may act as an inverse agonist possibly in a melanocortin-independent pathway that has yet-to-be determined. The intriguing finding that AgRP-deficient mice live significantly longer than controls, while consuming a high fat diet, opens up the possibility for AgRP to play a role in life expectancy. This adds a new dimension to the functional properties of AgRP and begs the question as to whether AgRP SNPs that have already been shown in humans to predispose to a leaner phenotype (a feature of longevity) may also predispose to a longer lifespan. AgRP could lie at the crossroads of energy balance regulation and aging, and our ability to reduce its expression levels could lead to a healthier and longer life.

References

1. Ollmann MM, Wilson BD, Yang YK, Kerns JA, Chen Y, Gantz I, Barsh GS. Antagonism of central melanocortin receptors in vitro and in vivo by agoutirelated protein. Science 1997;278:135–8. [PubMed: 9311920]
2. Shutter JR, Graham M, Kinsey AC, Scully S, Luthy R, Stark KL. Hypothalamic expression of ART, a novel gene related to agouti, is up-regulated in obese and diabetic mutant mice. Genes Dev 1997;11:593–602. [PubMed: 9119224]
3. Stutz AM, Morrison CD, Argyropoulos G. The Agouti-related protein and its role in energy homeostasis. Peptides 2005;26:1771–81. [PubMed: 15961186]
4. Vergoni AV, Bertolini A. Role of melanocortins in the central control of feeding. Eur. J. Pharmacol 2000;405:25–32. [PubMed: 11033311]
5. MacNeil DJ, Howard AD, Guan X, Fong TM, Nargund RP, Bednarek MA, Goulet MT, Weinberg DH, Strack AM, Marsh DJ, Chen HY, Shen CP, Chen AS, Rosenblum CI, MacNeil T, Tota M, MacIntyre ED, Van der Ploeg LH. The role of melanocortins in body weight regulation: opportunities for the treatment of obesity. Eur. J. Pharmacol 2002;440:141–57. [PubMed: 12007532]
6. Ellacott KL, Cone RD. The central melanocortin system and the integration of short- and long-term regulators of energy homeostasis. Recent Prog. Horm. Res 2004;59:395–408. [PubMed: 14749511]
7. Zimanyi IA, Pelleymounter MA. The role of melanocortin peptides and receptors in regulation of energy balance. Curr. Pharm. Des 2003;9:627–41. [PubMed: 12570796]
8. Graham M, Shutter JR, Sarmiento U, Sarosi I, Stark KL. Overexpression of Agrt leads to obesity in transgenic mice. Nat. Genet 1997;17:273–4. [PubMed: 9354787]
9. Bewick GA, Gardiner JV, Dhillo WS, Kent AS, White NE, Webster Z, Ghatei MA, Bloom SR. Post-embryonic ablation of AgRP neurons in mice leads to a lean, hypophagic phenotype. FASEB J 2005;19:1680–2. [PubMed: 16099943]
10. Gropp E, Shanabrough M, Borok E, Xu AW, Janoschek R, Buch T, Plum L, Balthasar N, Hampel B, Waisman A, Barsh GS, Horvath TL, Bruning JC. Agouti-related peptide-expressing neurons are mandatory for feeding. Nat. Neurosci 2005;8:1289–91. [PubMed: 16158063]
11. Luquet S, Perez FA, Hnasko TS, Palmiter RD. NPY/AgRP neurons are essential for feeding in adult mice but can be ablated in neonates. Science 2005;310:683–5. [PubMed: 16254186]
12. Mayfield DK, Brown AM, Page GP, Garvey WT, Shriver MD, Argyropoulos G. A role for the agouti related protein promoter in obesity and type 2 diabetes. Biochem. Biophys. Res. Commun 2001;287:568–573. [PubMed: 11554767]

13. Brown AM, Mayfield DK, Volaufova J, Argyropoulos G. The gene structure and minimal promoter of the human agouti related protein. Gene 2001;277:231–8. [PubMed: 11602360]

14. Beltramo M, Campanella M, Tarozzo G, Fredduzzi S, Corradini L, Forlani A, Bertorelli R, Reggiani A. Gene expression profiling of melanocortin system in neuropathic rats supports a role in nociception. Brain Res. Mol. Brain Res 2003;118:111–8. [PubMed: 14559360]

15. Halverson MJ, Donelan KJ, Granholm NH, Cheesbrough TM, Westby CA, Marshall DM. Rapid communication: sequencing of the porcine agouti-related protein (AGRP) gene. J. Anim. Sci 2002;80:1388–9. [PubMed: 12019634]

16. Adam CL, Archer ZA, Findlay PA, Thomas L, Marie M. Hypothalamic gene expression in sheep for cocaine- and amphetamine-regulated transcript, pro-opiomelanocortin, neuropeptide y, agouti-related Peptide and leptin receptor and responses to negative energy balance. Neuroendocrinology 2002;75:250–6. [PubMed: 11979055]

17. Klovins J, Haitina T, Fridmanis D, Kilianova Z, Kapa I, Fredriksson R, Gallo-Payet N, Schioth HB. The Melanocortin System in Fugu: Determination of POMC/AGRP/MCR Gene Repertoire and Synteny, As Well As Pharmacology and Anatomical Distribution of the MCRs. Mol. Biol. Evol 2004;21:563–79. [PubMed: 14694081]

18. Song Y, Golling G, Thacker TL, Cone RD. Agouti-related protein (AGRP) is conserved and regulated by metabolic state in the zebrafish, Danio rerio. Endocrine 2003;22:257–65. [PubMed: 14709799]

19. Cerda-Reverter JM, Peter RE. Endogenous melanocortin antagonist in fish: structure, brain mapping, and regulation by fasting of the goldfish agouti-related protein gene. Endocrinology 2003;144:4552–61. [PubMed: 12960082]

20. Boswell T, Li Q, Takeuchi S. Neurons expressing neuropeptide Y mRNA in the infundibular hypothalamus of Japanese quail are activated by fasting and co-express agouti-related protein mRNA. Brain Res. Mol. Brain Res 2002;100:31–42. [PubMed: 12008019]

21. Phillips-Singh D, Li Q, Takeuchi S, Ohkubo T, Sharp PJ, Boswell T. Fasting differentially regulates expression of agouti-related peptide, pro-opiomelanocortin, prepro-orexin, and vasoactive intestinal polypeptide mRNAs in the hypothalamus of Japanese quail. Cell. Tissue Res 2003;313:217–25. [PubMed: 12845520]

22. Strader AD, Buntin JD. Changes in agouti-related peptide during the ring dove breeding cycle in relation to prolactin and parental hyperphagia. J. Neuroendocrinol 2003;15:1046–53. [PubMed: 14622434]

23. Strader AD, Schioth HB, Buntin JD. The role of the melanocortin system and the melanocortin-4 receptor in ring dove (Streptopelia risoria) feeding behavior. Brain Res 2003;960:112–21. [PubMed: 12505663]

24. Hahn TM, Breininger JF, Baskin DG, Schwartz MW. Coexpression of Agrp and NPY in fasting-activated hypothalamic neurons. Nat. Neurosci 1998;1:271–2. [PubMed: 10195157]

25. Quillan JM, Jayawickreme CK, Lerner MR. Combinatorial diffusion assay used to identify topically active melanocyte-stimulating hormone receptor antagonists. Proc. Natl. Acad. Sci. USA 1995;92:2894–8. [PubMed: 7708744]

26. Quillan JM, Sadee W, Wei ET, Jimenez C, Ji L, Chang JK. A synthetic human Agouti-related protein-(83-132)-NH2 fragment is a potent inhibitor of melanocortin receptor function. FEBS Lett 1998;428:59–62. [PubMed: 9645475]

27. Yang YK, Dickinson CJ, Zeng Q, Li JY, Thompson DA, Gantz I. Contribution of melanocortin receptor exoloops to Agouti-related protein binding. J. Biol. Chem 1999;274:14100–6. [PubMed: 10318826]

28. Haskell-Luevano C, Cone RD, Monck EK, Wan YP. Structure activity studies of the melanocortin-4 receptor by in vitro mutagenesis: identification of agouti-related protein (agrp), melanocortin agonist and synthetic peptide antagonist interaction determinants. Biochemistry 2001;40:6164–79. [PubMed: 11352754]

29. 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. [PubMed: 11257308]

30. Thirumoorthy R, Holder JR, Bauzo RM, Richards NG, Edison AS, Haskell-Luevano C. Novel agouti-related-protein-based melanocortin-1 receptor antagonist. J. Med. Chem 2001;44:4114–24. [PubMed: 11708914]

31. Yang YK, Thompson DA, Dickinson CJ, Wilken J, Barsh GS, Kent SB, Gantz I. Characterization of Agouti-related protein binding to melanocortin receptors. Mol. Endocrinol 1999;13:148–55. [PubMed: 9892020]
32. Fong TM, Mao C, MacNeil T, Kalyani R, Smith T, Weinberg D, Tota MR, Van der Ploeg LH. ART (protein product of agouti-related transcript) as an antagonist of MC-3 and MC-4 receptors. Biochem. Biophys. Res. Commun 1997;237:629–31. [PubMed: 9299416]
33. Nijenhuis WA, Oosterom J, Adan RA. AgRP(83-132) acts as an inverse agonist on the human-melanocortin-4 receptor. Mol. Endocrinol 2001;15:164–71. [PubMed: 11145747]
34. Jackson PJ, McNulty JC, Yang YK, Thompson DA, Chai B, Gantz I, Barsh GS, Millhauser GL. Design, Pharmacology, and NMR Structure of a Minimized Cystine Knot with Agouti-Related Protein Activity. Biochemistry 2002;41:7565–7572. [PubMed: 12056887]
35. Millhauser GL, McNulty JC, Jackson PJ, Thompson DA, Barsh GS, Gantz I. Loops and links: structural insights into the remarkable function of the agouti-related protein. Ann. NY Acad. Sci 2003;994:27–35. [PubMed: 12851295]
36. 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. [PubMed: 12668211]
37. Creemers JW, Pritchard LE, Gyte A, Le Rouzic P, Meulemans S, Wardlaw SL, Zhu X, Steiner DF, Davies N, Armstrong D, Lawrence CB, Luckman SM, Schmitz CA, Davies RA, Brennand JC, White A. Agouti-related protein is posttranslationally cleaved by proprotein convertase 1 to generate agouti-related protein (AGRP)83-132: interaction between AGRP83-132 and melanocortin receptors cannot be influenced by syndecan-3. Endocrinology 2006;147:1621–31. [PubMed: 16384863]
38. Lee, M.; Kim, A.; Conwell, IM.; Wardlaw, SL. The Endocrine Society 88th Annual Meeting; The Endocrine Society Press, Boston, MA. 2006;
39. Goto K, Inui A, Takimoto Y, Yuzuriha H, Asakawa A, Kawamura Y, Tsuji H, Takahara Y, Takeyama C, Katsuura G, Kasuga M. Acute intracerebroventricular administration of either carboxyl-terminal or amino-terminal fragments of agouti-related peptide produces a long-term decrease in energy expenditure in rats. Int. J. Mol. Med 2003;12:379–83. [PubMed: 12883655]
40. Reizes O, Lincecum J, Wang Z, Goldberger O, Huang L, Kaksonen M, Ahima R, Hinkes MT, Barsh GS, Rauvala H, Bernfield M. Transgenic expression of syndecan-1 uncovers a physiological control of feeding behavior by syndecan-3. Cell 2001;106:105–16. [PubMed: 11461706]
41. Reizes O, Benoit SC, Strader AD, Clegg DJ, Akunuru S, Seeley RJ. Syndecan-3 modulates food intake by interacting with the melanocortin/AgRP pathway. Ann. NY Acad. Sci 2003;994:66–73. [PubMed: 12851299]
42. Schwartz MW, Baskin DG, Bukowski TR, Kuijper JL, Foster D, Lasser G, Prunkard DE, Porte D Jr. Woods SC, Seeley RJ, Weigle DS. Specificity of leptin action on elevated blood glucose levels and hypothalamic neuropeptide Y gene expression in ob/ob mice. Diabetes 1996;45:531–5. [PubMed: 8603777]
43. Broberger C, Johansen J, Johansson C, Schalling M, Hokfelt T. The neuropeptide Y/agouti gene-related protein (AGRP) brain circuitry in normal, anorectic, and monosodium glutamate-treated mice. Proc. Natl. Acad. Sci. USA 1998;95:15043–8. [PubMed: 9844012]
44. Wilson BD, Ollmann MM, Barsh GS. The role of agouti-related protein in regulating body weight. Mol. Med. Today 1999;5:250–6. [PubMed: 10366820]
45. Bruning JC, Gautam D, Burks DJ, Gillette J, Schubert M, Orban PC, Klein R, Krone W, Muller-Wieland D, Kahn CR. Role of brain insulin receptor in control of body weight and reproduction. Science 2000;289:2122–5. [PubMed: 11000114]
46. van den Top M, Lee K, Whyment AD, Blanks AM, Spanswick D. Orexigen-sensitive NPY/AgRP pace-maker neurons in the hypothalamic arcuate nucleus. Nat. Neurosci 2004;7:493–4. [PubMed: 15097991]
47. Bouret SG, Draper SJ, Simerly RB. Trophic action of leptin on hypothalamic neurons that regulate feeding. Science 2004;304:108–10. [PubMed: 15064420]
48. Minokoshi Y, Kahn BB. Role of AMP-activated protein kinase in leptin-induced fatty acid oxidation in muscle. Biochem. Soc. Trans 2003;31:196–201. [PubMed: 12546684]

49. Minokoshi Y, Alquier T, Furukawa N, Kim YB, Lee A, Xue B, Mu J, Foufelle F, Ferre P, Birnbaum MJ, Stuck BJ, Kahn BB. AMP-kinase regulates food intake by responding to hormonal and nutrient signals in the hypothalamus. Nature. 2004
50. Niswender KD, Gallis B, Blevins JE, Corson MA, Schwartz MW, Baskin DG. Immunocytochemical detection of phosphatidylinositol 3-kinase activation by insulin and leptin. J. Histochem. Cytochem 2003;51:275–83. [PubMed: 12588955]
51. Niswender KD, Morrison CD, Clegg DJ, Olson R, Baskin DG, Myers MG Jr. Seeley RJ, Schwartz MW. Insulin activation of phosphatidylinositol 3-kinase in the hypothalamic arcuate nucleus: a key mediator of insulin-induced anorexia. Diabetes 2003;52:227–31. [PubMed: 12540590]
52. Gao Q, Wolfgang MJ, Neschen S, Morino K, Horvath TL, Shulman GI, Fu XY. Disruption of neural signal transducer and activator of transcription 3 causes obesity, diabetes, infertility, and thermal dysregulation. Proc. Natl. Acad. Sci. USA 2004;101:4661–6. [PubMed: 15070774]
53. Bjorbaek C, Kahn BB. Leptin signaling in the central nervous system and the periphery. Recent Prog. Horm. Res 2004;59:305–31. [PubMed: 14749508]
54. Hegyi K, Fulop K, Kovacs K, Toth S, Falus A. Leptin-induced signal transduction pathways. Cell. Biol. Int 2004;28:159–69. [PubMed: 14984741]
55. Hakansson-Ovesjo ML, Collin M, Meister B. Down-regulated STAT3 messenger ribonucleic acid and STAT3 protein in the hypothalamic arcuate nucleus of the obese leptin-deficient (ob/ob) mouse. Endocrinology 2000;141:3946–55. [PubMed: 11089524]
56. Mesaros A, Koralov SB, Rother E, Wunderlich FT, Ernst MB, Barsh GS, Rajewsky K, Bruning JC. Activation of Stat3 Signaling in AgRP Neurons Promotes Locomotor Activity. Cell. Metab 2008;7:236–248. [PubMed: 18316029]
57. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 1999;402:656–60. [PubMed: 10604470]
58. Tschop M, Smiley DL, Heiman ML. Ghrelin induces adiposity in rodents. Nature 2000;407:908–13. [PubMed: 11057670]
59. Kamegai J, Tamura H, Shimizu T, Ishii S, Sugihara H, Wakabayashi I. Chronic central infusion of ghrelin increases hypothalamic neuropeptide Y and Agouti-related protein mRNA levels and body weight in rats. Diabetes 2001;50:2438–43. [PubMed: 11679419]
60. Kamegai J, Tamura H, Ishii S, Sugihara H, Wakabayashi I. Thyroid hormones regulate pituitary growth hormone secretagogue receptor gene expression. J. Neuroendocrinol 2001;13:275–8. [PubMed: 11207942]
61. Nakazato M, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K, Matsukura S. A role for ghrelin in the central regulation of feeding. Nature 2001;409:194–8. [PubMed: 11196643]
62. Wren AM, Small CJ, Abbott CR, Dhillo WS, Seal LJ, Cohen MA, Batterham RL, Taheri S, Stanley SA, Ghatei MA, Bloom SR. Ghrelin causes hyperphagia and obesity in rats. Diabetes 2001;50:2540–7. [PubMed: 11679432]
63. Kamegai J, Tamura H, Shimizu T, Ishii S, Sugihara H, Wakabayashi I. Central effect of ghrelin, an endogenous growth hormone secretagogue, on hypothalamic peptide gene expression. Endocrinology 2000;141:4797–800. [PubMed: 11108296]
64. Chen HY, Trumbauer ME, Chen AS, Weingarth DT, Adams JR, Frazier EG, Shen Z, Marsh DJ, Feighner SD, Guan XM, Ye Z, Nargund RP, Smith RG, Van Der Ploeg LH, Howard AD, MacNeil DJ, Qian S. Orexigenic Action of Peripheral Ghrelin is Mediated by Neuropeptide Y (NPY) and Agouti-Related Protein (AgRP). Endocrinology. 2004
65. Wortley KE, Anderson K, Garcia K, Murray J, Malinova L, Liu R, Moncrieffe M, Thabet K, Cox H, Yancopoulos GD, Wiegand SJ, Sleeman MW. Deletion of ghrelin reveals no effect on food intake, but a primary role in energy balance. Obes. Res 2004;12:170.
66. Drazen DL, Wortman MD, Schwartz MW, Clegg DJ, van Dijk G, Woods SC, Seeley RJ. Adrenalectomy alters the sensitivity of the central nervous system melanocortin system. Diabetes 2003;52:2928–34. [PubMed: 14633853]
67. Zakrzewska KE, Sainsbury A, Cusin I, Rouru J, Jeanrenaud B, Rohner-Jeanrenaud F. Selective dependence of intracerebroventricular neuropeptide Y-elicited effects on central glucocorticoids. Endocrinology 1999;140:3183–7. [PubMed: 10385413]

68. Madiehe AM, Lin L, White C, Braymer HD, Bray GA, York DA. Constitutive activation of STAT-3 and downregulation of SOCS-3 expression induced by adrenalectomy. Am. J. Physiol. Regul. Integr. Comp. Physiol 2001;281:R2048–58. [PubMed: 11705792]
69. Lu XY, Shieh KR, Kabbaj M, Barsh GS, Akil H, Watson SJ. Diurnal rhythm of agouti-related protein and its relation to corticosterone and food intake.PG-3905-15. Endocrinology 2002:143.
70. Jeanrenaud B, Rohner-Jeanrenaud F. CNS-periphery relationships and body weight homeostasis: influ- ence of the glucocorticoid status. Int. J. Obes. Relat. Metab. Disord 2000;24(Suppl 2):S74–6. [PubMed: 10997614]
71. Rocha M, Bing C, Williams G, Puerta M. Pregnancy-induced hyperphagia is associated with increased gene expression of hypothalamic agouti-related peptide in rats. Regul. Pept 2003;114:159–65. [PubMed: 12832105]
72. Sorensen A, Adam CL, Findlay PA, Marie M, Thomas L, Travers MT, Vernon RG. Leptin secretion and hypothalamic neuropeptide and receptor gene expression in sheep. Am. J. Physiol. Regul. Integr. Comp. Physiol 2002;282:R1227–35. [PubMed: 11893629]
73. Ge Y, Ohta T, Driscoll DJ, Nicholls RD, Kalra SP. Anorexigenic melanocortin signaling in the hypothalamus is augmented in association with failure-to-thrive in a transgenic mouse model for Prader-Willi syndrome. Brain Res 2002;957:42–5. [PubMed: 12443978]
74. Hillebrand JJ, Kas MJ, Scheurink AJ, van Dijk G, Adan RA. AgRP(83-132) and SHU9119 differently affect activity-based anorexia. Eur. Neuropsychopharmacol 2006;16:403–12. [PubMed: 16360312]
75. Marks DL, Cone RD. Central melanocortins and the regulation of weight during acute and chronic disease. Recent Prog. Horm. Res 2001;56:359–75. [PubMed: 11237221]
76. Xiao E, Xia-Zhang L, Vulliemoz NR, Ferin M, Wardlaw SL. Agouti-related protein stimulates the hypothalamic-pituitary-adrenal (HPA) axis and enhances the HPA response to interleukin-1 in the primate. Endocrinology 2003;144:1736–41. [PubMed: 12697678]
77. Dandona P, Aljada A, Bandyopadhyay A. Inflammation: the link between insulin resistance, obesity and diabetes. Trends Immunol 2004;25:4–7. [PubMed: 14698276]
78. Fekete C, Sarkar S, Rand WM, Harney JW, Emerson CH, Bianco AC, Lechan RM. Agouti-related protein (AGRP) has a central inhibitory action on the hypothalamic-pituitary-thyroid (HPT) axis; comparisons be- tween the effect of AGRP and neuropeptide Y on energy homeostasis and the HPT axis. Endocrinology 2002;143:3846–53. [PubMed: 12239096]
79. Graunke DM, Argyropoulos G. Deciphering Transcriptional Regulation Relevant to Eating Behavior. Curr. Genom 2003;4:623–633.
80. Bicknell AB, Lomthaisong K, Gladwell RT, Lowry PJ. Agouti related protein in the rat adrenal cortex: implications for novel autocrine mechanisms modulating the actions of pro- opiomelanocortin peptides. J. Neuroendocrinol 2000;12:977–82. [PubMed: 11012838]
81. Katsuki A, Sumida Y, Gabazza EC, Murashima S, Tanaka T, Furuta M, Araki-Sasaki R, Hori Y, Nakatani K, Yano Y, Adachi Y. Plasma levels of agouti-related protein are increased in obese men. J. Clin. Endocrinol. Metab 2001;86:1921–4. [PubMed: 11344185]
82. Shen CP, Wu KK, Shearman LP, Camacho R, Tota MR, Fong TM, Van Der Ploeg LH. Plasma agouti-related protein level: a possible correlation with fasted and fed States in humans and rats. J. Neuroendocrinol 2002;14:607–10. [PubMed: 12153462]
83. Kastin AJ, Akerstrom V, Hackler L. Agouti-related protein(83-132) aggregates and crosses the blood-brain barrier slowly. Metabolism 2000;49:1444–8. [PubMed: 11092509]
84. Claycombe KJ, Wang Y, Jones BH, Kim S, Wilkison WO, Zemel MB, Chun J, Moustaid-Moussa N. Transcriptional regulation of the adipocyte fatty acid synthase gene by agouti: interaction with insulin. Physiol. Genomics 2000;3:157–62. [PubMed: 11015611]
85. Claycombe KJ, Xue BZ, Mynatt RL, Zemel MB, Moustaid-Moussa N. Regulation of leptin by agouti. Physiol. Genomics 2000;2:101–5. [PubMed: 11015588]
86. Ebihara K, Ogawa Y, Katsuura G, Numata Y, Masuzaki H, Satoh N, Tamaki M, Yoshioka T, Hayase M, Matsuoka N, Aizawa-Abe M, Yoshimasa Y, Nakao K. Involvement of agouti-related protein, an endogenous antagonist of hypothalamic melanocortin receptor, in leptin action. Diabetes 1999;48:2028–33. [PubMed: 10512369]
87. Hoggard N, Hunter L, Duncan JS, Rayner DV. Regulation of adipose tissue leptin secretion by alpha-melanocyte-stimulating hormone and agouti-related protein: further evidence of an interaction

between leptin and the melanocortin signalling system. J Mol. Endocrinol 2004;32:145–53. [PubMed: 14765998]

88. Dhillo WS, Small CJ, Gardiner JV, Bewick GA, Whitworth EJ, Jethwa PH, Seal LJ, Ghatei MA, Hinson JP, Bloom SR. Agouti-related protein has an inhibitory paracrine role in the rat adrenal gland. Biochem. Biophys. Res. Commun 2003;301:102–7. [PubMed: 12535647]
89. Doghman M, Delagrange P, Blondet A, Berthelon MC, Durand P, Naville D, Begeot M. Agouti-Related Protein Antagonizes Glucocorticoid Production Induced through Mc4-R Activation in Bovine Adrenal Cells: A Possible Autocrine Control. Endocrinology. 2003
90. Takeuchi S, Takahashi S. A possible involvement of melanocortin 3 receptor in the regulation of adrenal gland function in the chicken. Biochim. Biophys. Acta 1999;1448:512–8. [PubMed: 9990303]
91. Takeuchi S, Teshigawara K, Takahashi S. Widespread expression of Agouti-related protein (AGRP) in the chicken: a possible involvement of AGRP in regulating peripheral melanocortin systems in the chicken. Biochim. Biophys. Acta 2000;1496:261–9. [PubMed: 10771094]
92. Korner J, Wissig S, Kim A, Conwell IM, Wardlaw SL. Effects of agouti-related protein on metabolism and hypothalamic neuropeptide gene expression. J. Neuroendocrinol 2003;15:1116–21. [PubMed: 14636173]
93. Backberg M, Madjid N, Ogren SO, Meister B. Down-regulated expression of agouti-related protein (AGRP) mRNA in the hypothalamic arcuate nucleus of hyperphagic and obese tub/tub mice. Brain Res. Mol. Brain Res 2004;125:129–39. [PubMed: 15193430]
94. Smith Richards BK, Belton BN, Poole AC, Mancuso JJ, Churchill GA, Li R, Volaufova J, Zuberi A, York B. QTL analysis of self-selected macronutrient diet intake: fat, carbohydrate, and total kilocalories. Physiol. Genomics 2002;11:205–17. [PubMed: 12388789]
95. York B, Lei K, West DB. Sensitivity to dietary obesity linked to a locus on chromosome 15 in a CAST/Ei x C57BL/6J F2 intercross. Mamm. Genome 1996;7:677–81. [PubMed: 8703121]
96. York B, Truett AA, Monteiro MP, Barry SJ, Warden CH, Naggert JK, Maddatu TP, West DB. Gene-environment interaction: a significant diet-dependent obesity locus demonstrated in a congenic segment on mouse chromosome 7. Mamm. Genome 1999;10:457–62. [PubMed: 10337618]
97. Breit A, Wolff K, Kalwa H, Jarry H, Buch T, Gudermann T. The natural inverse agonist agouti-related protein induces arrestin-mediated endocytosis of melanocortin-3 and -4 receptors. J. Biol. Chem. 2006
98. Tolle V, Low MJ. In vivo evidence for inverse agonism of agouti related peptide in the central nervous system of proopiomelanocortin deficient mice. Diabetes. 2007
99. Makimura H, Mizuno TM, Mastaitis JW, Agami R, Mobbs CV. Reducing hypothalamic AGRP by RNA interference increases metabolic rate and decreases body weight without influencing food intake. BMC Neurosci 2002;3:18. [PubMed: 12423556]
100. Xiang L, Murai A, Muramatsu T. The effects of agouti-related protein gene transfer in vivo by electroporation in mice. Neurosci. Lett 2004;370:108–13. [PubMed: 15488304]
101. Qian S, Chen H, Weingarth D, Trumbauer ME, Novi DE, Guan X, Yu H, Shen Z, Feng Y, Frazier E, Chen A, Camacho RE, Shearman LP, Gopal-Truter S, MacNeil DJ, Van Der Ploeg LH, Marsh DJ. Neither Agouti-Related Protein nor Neuropeptide Y Is Critically Required for the Regulation of Energy Homeostasis in Mice. Mol. Cell. Biol 2002;22:5027–35. [PubMed: 12077332]
102. Flier JS. AgRP in energy balance: Will the real AgRP please stand up? Cell. Metab 2006;3:83–5. [PubMed: 16459309]
103. Wortley KE, Anderson KD, Yasenchak J, Murphy A, Valenzuela D, Diano S, Yancopoulos GD, Wiegand SJ, Sleeman MW. Agouti-related protein-deficient mice display an age-related lean phenotype. Cell. Metab 2005;2:421–7. [PubMed: 16330327]
104. Redmann SM Jr. Argyropoulos G. AgRP-deficiency could lead to increased lifespan. Biochem. Biophys. Res. Commun 2006;351:860–864. [PubMed: 17097059]
105. Small CJ, Kim MS, Stanley SA, Mitchell JR, Murphy K, Morgan DG, Ghatei MA, Bloom SR. Effects of chronic central nervous system administration of agouti- related protein in pair-fed animals. Diabetes 2001;50:248–54. [PubMed: 11272133]
106. Wu Q, Howell MP, Cowley MA, Palmiter RD. Starvation after AgRP neuron ablation is independent of melanocortin signaling. Proc. Natl. Acad. Sci. USA 2008;105:2687–92. [PubMed: 18272480]

107. Fetissov SO, Bergstrom U, Johansen JE, Hokfelt T, Schalling M, Ranscht B. Alterations of arcuate nucleus neuropeptidergic development in contactin-deficient mice: comparison with anorexia and food-deprived mice. Eur. J. Neurosci 2005;22:3217–28. [PubMed: 16367788]

108. Johansen JE, Fetissov SO, Bergstrom U, Nilsson I, Fay C, Ranscht B, Hokfelt T, Schalling M. Evidence for hypothalamic dysregulation in mouse models of anorexia as well as in humans. Physiol. Behav 2007;92:278–82. [PubMed: 17560618]

109. Lachuer J, Ouyang L, Legras C, Del Rio J, Barlow C. Gene expression profiling reveals an inflammatory process in the anx/anx mutant mice. Brain Res. Mol. Brain Res 2005;139:372–6. [PubMed: 16006007]

110. Bai F, Rankinen T, Charbonneau C, Belsham DD, Rao DC, Bouchard C, Argyropoulos G. Functional dimorphism of two hAgRP promoter SNPs in linkage disequilibrium. J. Med. Genet 2004;41:350–3. [PubMed: 15121772]

111. Argyropoulos G, Rankinen T, Bai F, Rice T, Province M, Leon A, Skinner J, Wilmore J, Rao D, Bouchard B. The agouti related protein and body fatness in humans. International Journal of Obesity 2003;27:276–280. [PubMed: 12587010]

112. 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. Invest 2000;106:253–62. [PubMed: 10903341]

113. Vink T, Hinney A, van Elburg AA, van Goozen SH, Sandkuijl LA, Sinke RJ, Herpertz-Dahlmann BM, Hebebrand J, Remschmidt H, van Engeland H, Adan RA. Association between an agouti-related protein gene polymorphism and anorexia nervosa. Mol. Psychiatry 2001;6:325–8. [PubMed: 11326303]

114. Dubern B, Clement K, Pelloux V, Froguel P, Girardet JP, Guy-Grand B, Tounian P. Mutational analysis of melanocortin-4 receptor, agouti-related protein, and alpha-melanocyte-stimulating hormone genes in severely obese children. J. Pediatr 2001;139:204–9. [PubMed: 11487744]

115. Marks DL, Boucher N, Lanouette CM, Perusse L, Brookhart G, Comuzzie AG, Chagnon YC, Cone RD. Ala67Thr polymorphism in the Agouti-related peptide gene is associated with inherited leanness in humans. Am J. Med. Genet 2004;126 A:267–71. [PubMed: 15054840]

116. Tracy AL, Clegg DJ, Johnson JD, Davidson TL, Benoit SC. The melanocortin antagonist AgRP (83-132) increases appetitive responding for a fat, but not a carbohydrate, reinforcer. Pharmacol. Biochem. Behav. 2007

117. Sozen MA, de Jonge LH, Greenway F, Ravussin E, Smith SR, Argyropoulos G. A rare mutation in AgRP, +79G>A, affects promoter activity. Eur. J. Clin. Nutr. 2006

118. Hoggard N, Rayner DV, Johnston SL, Speakman JR. Peripherally administered [Nle4,D-Phe7]-alpha-melanocyte stimulating hormone increases resting metabolic rate, while peripheral agouti-related protein has no effect, in wild type C57BL/6 and ob/ob mice. J Mol. Endocrinol 2004;33:693–703. [PubMed: 15591028]

119. Gavrila A, Chan JL, Miller LC, Heist K, Yiannakouris N, Mantzoros CS. Circulating melanin-concentrating hormone, agouti-related protein, and alpha-melanocyte-stimulating hormone levels in relation to body composition: alterations in response to food deprivation and recombinant human leptin administration. J. Clin. Endocrinol. Metab 2005;90:1047–54. [PubMed: 15546902]

120. Rossi M, Kim MS, Morgan DG, Small CJ, Edwards CM, Sunter D, Abusnana S, Goldstone AP, Russell SH, Stanley SA, Smith DM, Yagaloff K, Ghatei MA, Bloom SR. A C-terminal fragment of Agouti-related protein increases feeding and antagonizes the effect of alpha-melanocyte stimulating hormone in vivo. Endocrinology 1998;139:4428–31. [PubMed: 9751529]

121. Hagan MM, Rushing PA, Pritchard LM, Schwartz MW, Strack AM, Van Der Ploeg LH, Woods SC, Seeley RJ. Long-term orexigenic effects of AgRP-(83-132) involve mechanisms other than melanocortin receptor blockade. Am. J. Physiol. Regul. Integr. Comp. Physiol 2000;279:R47–52. [PubMed: 10896863]

122. Rosenfeld RD, Zeni L, Welcher AA, Narhi LO, Hale C, Marasco J, Delaney J, Gleason T, Philo JS, Katta V, Hui J, Baumgartner J, Graham M, Stark KL, Karbon W. Biochemical, biophysical, and pharmacological characterization of bacterially expressed human agouti-related protein. Biochemistry 1998;37:16041–52. [PubMed: 9819197]

123. Small CJ, Liu YL, Stanley SA, Connoley IP, Kennedy A, Stock MJ, Bloom SR. Chronic CNS administration of Agouti-related protein (Agrp) reduces energy expenditure. Int. J. Obes. Relat. Metab. Disord 2003;27:530–3. [PubMed: 12664087]

124. Kim MS, Rossi M, Abbott CR, AlAhmed SH, Smith DM, Bloom SR. Sustained orexigenic effect of Agouti related protein may be not mediated by the melanocortin 4 receptor. Peptides 2002;23:1069–76. [PubMed: 12126733]

125. Zheng H, Corkern MM, Crousillac SM, Patterson LM, Phifer CB, Berthoud HR. Neurochemical phenotype of hypothalamic neurons showing Fos expression 23 h after intracranial AgRP. Am. J. Physiol. Regul. Integr. Comp. Physiol 2002;282:R1773–81. [PubMed: 12010760]

126. Nilsson I, Lindfors C, Fetissov SO, Hokfelt T, Johansen JE. Aberrant agouti-related protein system in the hypothalamus of the anx/anx mouse is associated with activation of microglia. J. Comp. Neurol 2008;507:1128–40. [PubMed: 18098136]

127. Argyropoulos G, Rankinen T, Neufeld DR, Rice T, Province MA, Leon AS, Skinner JS, Wilmore JH, Rao DC, Bouchard C. A polymorphism in the human agouti-related protein is associated with late-onset obesity. J. Clin. Endocrinol. Metab 2002;87:4198–202. [PubMed: 12213871]

Table 1

Phenotypes of AgRP mouse models

MODEL	PHENOTYPE	REFERENCE
AgRP transgenic	Food intake ↑, body weight ↑, body length ↑, corticosterone levels ↓	[8]
i.c.v. injection of AgRP (aa87-132)	Food intake ↑, body weight ↑, energy expenditure ↓	[23,39,76,120-125]
AgRP k.o.	Normal weight gain and feeding behavior, slight difference in MCH	[64,101]
AgRP/NPY double k.o.	Normal weight gain and feeding behavior, unresponsive to ghrelin administration	[64,101]
AgRP k.o.	Age-related lean phenotype	[103]
AgRP k.o.	Increased lifespan under high fat diet	[104]
AgRP RNAi injection into hypothalamus	50% inhibition of AGRP expression, metabolic rate ↑, body weight ↓, normal food intake	[99]
AgRP overexpression in leg	Food intake ↑Body weight ↑	[100]
Ablation of AgRP neurons	Hypophagic phenotype Essential for feeding in adult mice	[9,11]
anx/anx	Anorexia Defective AgRP projections Short-lived (3-5 weeks)	[108,109,126]
Contactin k.o.	Anorexia Defective AgRP projections Short-lived (<3 weeks)	[107,108]

↑increase

↓decrease

Table 2

Polymorphisms in the human AgRP gene and their associated phenotypes

SNP	FUNCTION	PHENOTYPE	REFERENCE
-3019G>A	Alters promoter activity	In complete LD with -38C>T (found in Blacks only)	[110]
-38C>T	Alters promoter activity	Associated with reduced obesity and T2D (found in Blacks only)	[12,111]
+79G>A	Alters promoter activity in adrenal but not neuronal cells	Could predispose to decreased resting metabolic rate (rare, found in two Whites only)	[117]
G423A	Silent	None reported	[112]
C690T	Silent	None reported	[112]
Ala67Thr	Alters secondary structure (algorithm prediction)	Associated with: anorexia nervosa, resistance to late-onset obesity, preference for carbohydrates (found in Whites only)	[13,113-115,127]