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Lack of Proopiomelanocortin Peptides Results in Obesity and Defective Adrenal Function but Normal Melanocyte Pigmentation in the Murine C57BL/6 Genetic Background

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ABSTRACT: Mice deficient in proopiomelanocortin peptides (*Pomc*^{-/-}) generated on a 129 (*A^w/A^w*) genetic background were back-crossed onto the C57BL/6 (*a/a*) genetic background. These mice exhibited most of the phenotypic characteristics previously reported on the 129 genetic background (Yaswen *et al.* 1999, Nat. Med. 5: 1066–1070). Adult mice became obese, their adrenals were atrophied, and they had undetectable plasma corticosterone in basal and stressed states. The partial perinatal lethality previously reported was also present on the C57BL/6 background. In addition, we found that both male and female homozygote (-/-) adults were fertile, but when homozygous males were intercrossed with homozygous females, all the pups died in the perinatal period. Attempts to rescue the perinatal lethality of pups from homozygous breeder pairs by supplementing the mother's drinking water with glucocorticoids were unsuccessful. Furthermore, failure to stimulate adrenal development and corticosterone production/release with daily exogenous adrenocorticotropin-stimulating hormone (ACTH) injections indicates an adrenal dependence on POMC peptides for normal development and function. While the original *Pomc*^{-/-} mice, bred on a mixed white-bellied agouti (*A^w/A^w*) 129 genetic background, had patchy alternations in their coat color, they clearly were not a uniform yellow like the *lethal yellow* (*A^y/a*) mice. Our *Pomc*^{-/-} mice bred onto the C57BL/6 (*a/a*) genetic background had a black coat color indistinguishable from that of the wild-type C57BL/6 mice, further suggesting that the POMC peptide melanocyte-stimulating hormone (α -MSH) is not essential for the production of eumelanin (black/brown) pigmentation.

KEYWORDS: proopiomelanocortin (POMC); obesity; adrenal; hypothalamic-pituitary-adrenal axis (HPA); corticosterone; pigmentation

INTRODUCTION

Over the last ten years, the role of proopiomelanocortin (POMC) peptides as central regulators of energy homeostasis has become very apparent.¹ Early evidence centered on the detection of alpha-melanocyte-stimulating hormone (α -MSH) bind-

ing to hypothalamic nuclei involved in regulating energy homeostasis.² Since then, a variety of mutant mouse models have shown central α -MSH to be an anorexigenic peptide, whereas the POMC opioid β -endorphin may play a more complex role in a reward pathway that reinforces feeding behavior.^{3–7} In addition to the energy homeostatic role of central POMC peptides, a large body of evidence implicates central POMC involvement in stress responses, reproduction, and ontogeny of the hypothalamus, pituitary, and adrenal glands.^{8–14} However, further experiments are needed to conclusively establish these hypothesized roles of central POMC peptides from other peptidergic systems. Additionally, *in vivo* experiments using POMC transgenes are currently underway to delineate the roles of central POMC peptides from those of peripheral POMC peptides released from the pituitary gland corticotrophs and melanotrophs. This manuscript identifies specific physiological alterations in the *Pomc*^{−/−} mutant mice on the C57BL/6 genetic background.

ORIGINAL POMC NULL MUTATION

A mouse strain deficient in all POMC peptides was generated by the Hochgeschwender and Brennan laboratories using homologous recombination of a replacement-type *Pomc* allele containing a deletion of exon 3 and insertion of *neo*. This mutant strain was generated on a mixed white-bellied agouti 129 genetic background (*A^w/A^w pTyr^C/pTyr^{C-Ch}*) and develops hyperphagia and obesity similar to those seen in the *lethal yellow* (*A^y/a*) mice and melanocortin receptor-4 (MC4-R)–deficient mice.¹⁵ However, in contrast to these and other previously described obesity models, *Pomc*^{−/−} mice have no detectable levels of circulating corticosterone or epinephrine. Analysis of adult suprarenal fat pads revealed what appeared to be an adrenal remnant but no clearly identifiable adrenal glands. A possible result of the adrenal absence was the partial perinatal lethality seen in offspring of heterozygous breeder pairs. The loss of circulating α -MSH, the melanocortin receptor-1 (MC1-R) agonist, was predicted to eliminate the production of eumelanin (black/brown) pigmentation in melanocytes of the *Pomc*^{−/−} mice; however, they had only modest increases in pheomelanin (yellow) pigmentation, most noticeable on their bellies.

INCREASED ADIPOSITY AND LINEAR GROWTH

To better understand the contribution of POMC peptides to hair follicle pigmentation, we back-crossed the *Pomc*^{−/−} mutant allele from the agouti (*A^w/A^w*) 129 genetic background on to the C57BL/6 (*a/a*) genetic background for two successive generations. Additionally, the C57BL/6 genetic background allows us to study proposed POMC physiological functions on a more commonly used inbred mouse line and one that is predisposed to diet-induced obesity, thus eliminating confounds due to genetic heterogeneity when comparing data to the melanocortin receptor knock-out and other spontaneous mouse obesity models.

Analysis of growth curves of *Pomc*^{−/−} mice (FIG. 1) revealed an increase in body mass very similar to that seen in the *lethal yellow* (*A^y/a*) and MC4-R–deficient mice.^{3,16} Further analysis showed that the increased size of the *Pomc*^{−/−} mice was due to both increased linear growth and increased mass of white fat depots, again

FIGURE 1. Male and female mice were group housed, fed a standard diet of rodent chow (5% fat, 19% protein, and 5% fiber by weight; 3.4 kcal/g), and weighed monthly. *Pomc*^{-/-} mice became significantly heavier than wild-type littermates at 6–8 weeks old for males and 4–6 weeks old for females. Heterozygous males developed later onset obesity at about 8–10 months, whereas heterozygous females had a smaller increase in body mass at 8–10 months. (○, homozygous; △, heterozygous; □, wild-type; *N* = 25 to 105.)

similar to those of the *lethal yellow* and MC4-R-deficient mice. There were no obvious differences in the growth curves of *Pomc*^{-/-} mice on the 129 versus C57BL/6 genetic background. However, all murine mutant models besides the *Pomc*^{-/-} mice have circulating glucocorticoids. In addition, when the *lethal yellow* or the *leptin-deficient ob/ob* mice are adrenalectomized, these mice lose a significant amount of their white-fat mass, suggesting that glucocorticoids play a significant role in the development of obesity in these mutant mouse models.¹⁶ *Pomc*^{-/-} mice develop an obesity phenotype in the absence of circulating glucocorticoids, suggesting a role for POMC peptides regulating energy homeostasis independent of corticosterone.

ADRENAL ATROPHY AND HORMONE DEFICIENCIES

The adrenal gland, composed of an outer cortical shell and an inner medullary core, secretes the steroid hormones corticosterone, aldosterone, and androgens together with the catecholamines norepinephrine and epinephrine. Although the *Pomc*^{-/-} mutant mice on the 129 genetic background fail to develop any distinguishable adrenal glands, the C57BL/6 mutant mice have easily identifiable, but severely hypoplastic adrenals. *Pomc*^{-/-} mice adrenal glands were approximately 15% (in females) to 30% (in males) the weight of their respective wild-type littermates (FIG. 2). The greater reduction in the size of female adrenal glands compared with male adrenal glands eliminates the sexual dimorphism normally found in wild-type

FIGURE 2. Adrenals were removed, trimmed of fat, and weighed as a pair from each mouse. Male *Pomc*^{-/-} mice adrenals were 70% smaller than those from their wild-type and heterozygous littermates. Female *Pomc*^{-/-} mice adrenals were 85% smaller than those from their wild-type and heterozygous littermates. In addition, female *Pomc*^{+/-} heterozygous mice adrenals were slightly, but significantly, smaller than those from wild-type littermates. (***) $P < 0.0001$ and (**) $P < 0.01$ compared to wild-type; $N = 5$ to 15.)

mice. Despite the presence of these hypoplastic adrenals on the C57BL/6 genetic background, plasma collected from unstressed, restraint-stressed, or lipopolysaccharide-challenged *Pomc*^{-/-} mice still had no detectable levels of corticosterone (data not shown). Furthermore, treatment of *Pomc*^{-/-} mice with 1 μ g adrenocorticotropin-stimulating hormone (ACTH) (1–24) twice daily for 2 weeks failed to elicit any corticosterone secretory response, whereas wild-type mice responded promptly to the same dose of exogenous ACTH 3 h after dexamethasone suppression of their hypothalamic-pituitary-adrenal (HPA) axis (FIG. 3). The adrenal deficiencies in these mice strengthen the hypothesis that normal POMC expression is needed for complete adrenal maturation; however, the role of NH₂-terminal peptides in addition to ACTH remains an open question.¹⁰

We hypothesize that the lack of adrenal hormones, and not POMC peptides *per se*, is responsible for the non-Mendelian production of viable *Pomc*^{-/-} offspring (FIG. 4). Both heterozygous and homozygous male and female mice were shown to be fertile. Further evaluation of heterozygous breeder pairs revealed that transmission of the *Pomc*^{-/-} allele was normal but that 50–70% of the homozygous *Pomc*^{-/-} mice die in the perinatal period, typically within the first hour after birth. Homozygous breeder pairs produced full-term litters with no pup surviving the perinatal period. Mice lacking corticotrophin-releasing hormone (CRH) are also unable to make corticosterone and exhibit a similar perinatal lethality.¹⁷ The CRH (-/-) lethality can be rescued by administration of glucocorticoids to the mother's drinking water. However, an identical attempt to rescue the lethality in the homozygous *Pomc*^{-/-} breeder pairs proved unsuccessful, suggesting the *Pomc*^{-/-} perinatal lethality may be a result of the combined lack of glucocorticoids and epinephrine in the

FIGURE 3. EDTA-plasma corticosterone levels were measured using a ^{125}I -labeled corticosterone radioimmunoassay kit from ICN. Dexamethasone ($10\text{ }\mu\text{g}/\text{mouse}$) was given ip immediately following blood draw at baseline time point A. At all other time points, mice received either saline or $1\text{ }\mu\text{g}$ ACTH ($1\text{--}24$) ip 30 min prior to blood collection from a tail vein. All null male mouse EDTA-plasma samples at all time points were below the assay sensitivity (25 ng/mL). (A) Dexamethasone injection (time 0). (B) 2.5 h after dexamethasone (saline or ACTH injection). (C) 3.0 h after dexamethasone and 30 min after saline or ACTH. (D) 7 days after twice daily ACTH injections ($1\text{ }\mu\text{g}/\text{injection}$). (E) 14 days after twice daily ACTH injections ($1\text{ }\mu\text{g}/\text{injection}$). (***) $P < 0.001$ compared to baseline values at point A; $N = 2$ to 6.)

Breeder Pair:	(+/-) X (+/-)		
Offspring:	(+/+)	(+/-)	(-/-)
Number:	83	158	31
Percentage:	30.5%	58.1%	11.4%

Breeder Pair:	(-/-) X (+/-)		
Offspring:	(+/+)	(+/-)	(-/-)
Number:	0	93	27
Percentage:	0%	77.5%	22.5%

FIGURE 4. Offspring numbers and percentages of total offspring for each genotype are shown for two types of breeder pairs: heterozygous *Pomc*^{+/-} male X heterozygous *Pomc*^{+/-} female, and homozygous *Pomc*^{-/-} male X heterozygous *Pomc*^{+/-} female.

Pomc^{-/-} pups. *Pomc*^{-/-} mice expressing a transgene that rescues pituitary POMC peptides and therefore adrenal corticosterone production, but that are still void of central POMC peptides are currently being evaluated.

PIGMENTATION IS UNALTERED IN THE C57BL/6 GENETIC BACKGROUND

Information gained from the *recessive yellow* (*Mc1r*^l/*Mc1r*^l) and the autosomal-dominant *lethal yellow* (*A*^y/*a*) mice would predict an inability to make eumelanin pigmentation in the *Pomc*^{-/-} mice.¹⁸ This prediction for the *Pomc*^{-/-} mice is based on the absence of stimulation of the MC1-R by its natural POMC peptide agonist, α -MSH. In the *lethal yellow* mice, the constitutively activated expression of agouti-ipeptide antagonizes the MC1-R, preventing stimulation by α -MSH and inhibiting eumelanin synthesis. The original *Pomc*^{-/-} mice on an agouti (*A*^w/*A*^w) 129 genetic background resulted in only subtle alteration of yellow hair pigmentation on the belly, much different from the coat color seen in the *lethal yellow* (*A*^y/*a*) mice. The presence of agouti in the 129 genetic background produces a banded hair shaft with both eumelanin and phaeomelanin pigmentation, complicating the analysis of coat color on this genetic background. To more closely evaluate the potential alterations in coat color in the absence of peripheral α -MSH, the *Pomc*^{-/-} allele was evaluated on an agouti-less genetic background, C57BL/6 (*a/a*), to eliminate all phaeomelanin caused by antagonism of α -MSH binding. Our studies show no distinguishable difference in *Pomc*^{-/-} black coat color from wild-type (+/+) or heterozygous (+/-)

FIGURE 5. (A) Coronal vibratome sections (50- μ m thick) from a compound *Pomc*^{-/-}, *Pomc*-EGFP^{Tg/+} mouse were viewed under UV light at 10 $\times$ magnification on a Zeiss microscope with an FITC filter cube. Neurons expressing EGFP are brightly fluorescent and distributed in the arcuate nucleus of the basal hypothalamus in the same location as POMC neurons. (B) A pituitary section (12 μ m) exhibiting robust fluorescence in the intermediate lobe (IL) and the anterior lobe (AL) under UV light at 3 $\times$ magnification. Fluorescent cells seen in both intermediate and anterior lobes closely resemble those seen in melanotroph and corticotroph distributions.

littermates on the C57BL/6 background, including the presence of a few yellow hairs behind the ears, in the perianal area and around the female nipples seen on wild-type C57BL/6 mice. Possible explanations for the black coat color in the absence of α -MSH include an endogenous constitutively active MC1-R, compensatory pathways that stimulate cAMP production and tyrosinase activity, thereby bypassing the MC1-R altogether, and alternative endogenous agonist ligands for the receptor.

POMC NEURONS, CORTICOTROPHS, AND MELANOTROPHS ARE STILL PRESENT IN *Pomc*^{-/-} MICE

The inability to immunohistochemically detect POMC cells in *Pomc*^{-/-} mice makes it difficult to know if the cells, void of the POMC peptides, are still present. To identify and study POMC neurons *in vivo*, we previously generated a transgenic C57BL/6 mouse line expressing enhanced green fluorescent protein (EGFP, Clontech) under the transcriptional control of POMC genomic regulatory elements.¹⁹ The transgene was designed to only express EGFP under the *Pomc* promoter, but not to express any of the POMC peptides. We back-crossed this POMC-EGFP transgene onto the *Pomc*^{-/-} mice to determine if the POMC neurons and POMC pituitary cells developed in their normal numbers and locations. Our analysis revealed that POMC neurons along with pituitary corticotrophs and melanotrophs remain intact in the *Pomc*^{-/-} mice (FIG. 5). POMC neurons have been found to coexpress another peptide called cocaine-amphetamine-regulated transcript (CART); however, no other peptide has been shown to be coexpressed in corticotrophs and melanotrophs with

POMC peptides.²⁰ Whether CART neuron physiology has been altered and/or whether pituitary corticotrophs and melanotrophs void of POMC peptides have any physiological function in the *Pomc*^{-/-} mouse has yet to be determined.

CONCLUSION

The heterogeneity found among the many inbred mouse lines can result in an epistatic modification of phenotypes when the same genetic mutations are analyzed.^{21,22} Therefore, the back-crossing and analysis of the *Pomc*^{-/-} allele are essential for understanding the function of POMC peptides on the C57BL/6 genetic background, an inbred strain that has been used for functional analysis of melanocortin-receptor-deficient mice and other spontaneous obesity models. In addition, C57BL/6 mice are sensitive to diet-induced obesity and have been the subject of extensive behavioral and energy homeostatic studies. Although much of the data originally obtained from the mutant POMC allele on the 129 genetic background has been recapitulated in the C57BL/6 genetic background, we have found certain discrepancies in the phenotypes. Most notably, we find that the coat color of *Pomc*^{-/-} on the C57BL/6 background is indistinguishable from that of wild-type littermates. In addition, we find that the adrenals are present in adult *Pomc*^{-/-} mice on the C57BL/6 background, although they are very much reduced in size and still incapable of secreting corticosterone or responding to exogenous ACTH stimulation.

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REFERENCES

1. ELMQUIST, J.K., C.F. ELIAS & C.B. SAPER. 1999. From lesions to leptin: hypothalamic control of food intake and body weight. *Neuron* **22**: 221–232.
2. TATRO, J.B. 1990. Melanotropin receptors in the brain are differentially distributed and recognize both corticotropin and alpha-melanocyte stimulating hormone. *Brain Res.* **536**: 124–132.
3. HUSZAR, D. *et al.* 1997. Targeted disruption of the melanocortin-4 receptor results in obesity in mice. *Cell* **88**: 131–141.
4. BUTLER, A.A. *et al.* 2000. A unique metabolic syndrome causes obesity in the melanocortin-3 receptor-deficient mouse. *Endocrinology* **141**: 3518–3521.
5. CHEN, A.S. *et al.* 2000. Inactivation of the mouse melanocortin-3 receptor results in increased fat mass and reduced lean body mass. *Nat. Genet.* **26**: 97–102.
6. APPELEYARD, S.M. *et al.* 2003. A role for the endogenous opioid β -endorphin in energy homeostasis. *Endocrinology* **144**: 1753–1760.
7. HAYWARD, M.D., J.E. PINTAR & M.J. LOW. 2002. Selective reward deficit in mice lacking beta-endorphin and enkephalin. *J. Neurosci.* **22**: 8251–8258.
8. ALLEN, R.G. *et al.* 1984. Biosynthesis and processing of pro-opiomelanocortin-derived peptides during fetal pituitary development. *Dev. Biol.* **102**: 43–50.

9. BERRY, S. & L.W. HAYNES. 1989. The opiomelanocortin peptide family: neuronal expression and modulation of neural cellular development and regeneration in the central nervous system. *Comp. Biochem. Physiol. A* **93**: 267–272.
10. BICKNELL, A.B. *et al.* 2001. Characterization of a serine protease that cleaves pro-gamma-melanotropin at the adrenal to stimulate growth. *Cell* **105**: 903–912.
11. ESTIVARIZ, F.E. *et al.* 1988. Adrenal regeneration in the rat is mediated by mitogenic N-terminal pro-opiomelanocortin peptides generated by changes in precursor processing in the anterior pituitary. *J. Endocrinol.* **116**: 207–216.
12. ESTIVARIZ, F.E. *et al.* 1988. Further evidence that N-terminal pro-opiomelanocortin peptides are involved in adrenal mitogenesis. *J. Endocrinol.* **116**: 201–206.
13. MARSH, D.J. *et al.* 1999. Response of melanocortin-4 receptor-deficient mice to anorectic and orexigenic peptides. *Nat. Genet.* **21**: 119–122.
14. ZAGON, I.S. & P.J. McLAUGHLIN. 1987. Endogenous opioid systems regulate cell proliferation in the developing rat brain. *Brain Res.* **412**: 68–72.
15. YASWEN, L. *et al.* 1999. Obesity in the mouse model of pro-opiomelanocortin deficiency responds to peripheral melanocortin. *Nat. Med.* **5**: 1066–1070.
16. BOSTON, B.A. *et al.* 1997. Independent and additive effects of central POMC and leptin pathways on murine obesity. *Science* **278**: 1641–1644.
17. MUGLIA, L. *et al.* 1995. Corticotropin-releasing hormone deficiency reveals major fetal but not adult glucocorticoid need. *Nature* **373**: 427–432.
18. OLLMANN, M.M. *et al.* 1998. Interaction of Agouti protein with the melanocortin 1 receptor in vitro and in vivo. *Genes Dev.* **12**: 316–330.
19. COWLEY, M.A. *et al.* 2001. Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. *Nature* **411**: 480–484.
20. ELIAS, C.F. *et al.* 1998. Leptin activates hypothalamic CART neurons projecting to the spinal cord. *Neuron* **21**: 1375–1385.
21. BUTLER, A.A. & R.D. CONE. 2002. The melanocortin receptors: lessons from knockout models. *Neuropeptides* **36**: 77–84.
22. KOZAK, L.P. & M. ROSSMEISL. 2002. Adiposity and the development of diabetes in mouse genetic models. *Ann. N.Y. Acad. Sci.* **967**: 80–87.