60 YEARS OF POMC

The proopiomelanocortin gene: discovery, deletion and disease

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Abstract

The cloning of the bovine proopiomelanocortin (*POMC*) cDNA in 1978 by Nakanishi and colleagues was the result of a remarkable series of exacting and ingenious experiments. With this work, they instantly confirmed the single precursor hypothesis for adrenocorticotrophic hormone-β-lipotropin, as it was then known, and in so doing revealed the existence of additional, largely unpredicted, N-terminal peptides that together formed the POMC precursor peptide. This work paved the way for a host of additional studies into the physiology of these peptides and their regulation. Furthermore, the cloning of the murine *Pomc* gene was essential for subsequent studies, in which *Pomc* was intentionally deleted in the mouse illuminating its substantial role in body weight regulation and adrenal function. Contemporaneously with this work, naturally occurring mutations in human *POMC* came to light underlining the vital role of this gene in appetite regulation. This article reviews each of these aspects of *POMC* with the benefit of several decades of hindsight and informed by more recent genomic and transcriptomic data.

Key Words
- proopiomelanocortin
- adrenocorticotrophin
- adrenal
- obesity
- genetic disease

Journal of Molecular Endocrinology
(2016) **56**, T27–T37

Journal of Molecular Endocrinology

Introduction

The concept that one precursor peptide, adrenocorticotrophic hormone-β-lipotropin (ACTH-β-LPH), could contain more han one biologically active component had become clear by the mid-1970s as other papers in this issue have described. However, a true understanding of the precise relationships of these peptides and an independent confirmation of the common precursor hypothesis required the isolation of the gene encoding ACTH-β-LPH.

While this might seem an almost trivial exercise today when we have access to techniques such as the PCR and high-throughput sequencing, and the availability of high-quality enzymes and other molecular biology reagents, this was not the case in the mid-1970s. At this time, although the techniques of gene and cDNA cloning had become reasonably well established, this undertaking remained a significant challenge.

Gene cloning

Nakanishi and Numa in Kyoto led the way in this task in collaboration with the Cohen group at Stanford, USA as described in a series of landmark papers. In particular, they exploited the techniques of cell-free or *in vitro* translation using either wheat germ extracts or rabbit reticulocyte lysates. Using mRNA from 20 bovine pituitaries, they were able to translate the proteins encoded by this RNA and to show that a single protein species could be precipitated with two antibodies raised against distinct epitopes of the

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DOI: 10.1530/JME-15-0268

This paper is part of a thematic review section on 60 Years of POMC.
The Guest Editors for this section were Adrian Clark and Philip Lowry.

ACTH molecule (Nakanishi *et al.* 1976). Liver mRNA when translated contained none of this protein, as expected. They went on to show that this 41 kDa protein could be precipitated from either bovine anterior pituitary or neurointermediate lobe by both ACTH and β-endorphin antibodies, providing strong support to the single precursor hypothesis (Nakanishi *et al.* 1977). It emerged that the neurointermediate lobe was a particularly rich source of this mRNA, and that about 30% of mRNA from this tissue encoded the ACTH/β-LPH precursor (Taii *et al.* 1979). Using sucrose density gradient centrifugation of membrane-bound mRNA (i.e. polysome associated), a single species of mRNA of approximately 1360 nucleotides in length was purified (Kita *et al.* 1979).

Conversion of this purified mRNA into double-stranded cDNA was followed by ligation into a plasmid carrying an antibiotic resistance gene and transformation of bacteria. Individual antibiotic-resistant colonies were selected and the presence of ACTH-β-LPH mRNA confirmed by hybridization with ^{32}P-labeled neurointermediate lobe mRNA. Twelve independent cDNA clones were selected of which the largest contained a cDNA of 1120 bp in length; potentially sufficient to encode the entire protein precursor (Nakanishi *et al.* 1978).

This was subjected to DNA sequencing and provided the first cDNA sequence for the ACTH-β-LPH precursor, thus independently confirming the existence of a large precursor protein (Nakanishi *et al.* 1979). The cloned cDNA encoded a translation product containing a putative N-terminal secretory signal sequence of 26 amino acids, a novel peptide fragment lying N-terminal to the ACTH sequence, the ACTH sequence itself, and at the C-terminus of the β-LPH sequence terminating in a stop codon. The novel N-terminal segment contained within it a previously unrecognized melanocyte-stimulating hormone (MSH)-like sequence, containing the characteristic MSH signature sequence (His-Phe-Arg-Trp), which was tentatively named γ-MSH. All of the putative peptides were flanked by dibasic amino acids (or a stop codon at the C-terminus of β-LPH), a finding which at that time had also been observed in certain other peptide precursors. The nature of the N-terminus and the identity of the initiator methionine was independently confirmed by protein sequencing of the *in vitro* translated protein product (Nakamura *et al.* 1979). These findings are summarized in Fig. 1. Cloning of related cDNAs from the pig and mouse were reported subsequently (Boileau *et al.* 1983, Uhler & Herbert 1983). The term proopiomelanocortin (POMC) was first coined by Chrétien and coworkers and will be used hereafter (Chrétien *et al.* 1979).

Genomic DNA

The availability of the cloned full-length *POMC* cDNA was used to screen genomic DNA libraries of both bovine and human origin. This led in a series of publications to the identification of the complete gene structure of human and bovine *POMC* (Chang *et al.* 1980, Nakanishi *et al.* 1981, Takahashi *et al.* 1981, Whitfeld *et al.* 1983). The gene in both species consisted of three exons. Exon 1 was 87 bp long (in the human) and contained no translation product. This was followed by a 4 kb intron upstream of exon 2. This short second exon (152 bp) encoded the initiator methionine and signal sequence before intron 2 of 2.2 kb (Nakanishi *et al.* 1980). The majority of the coding region was contained within the largest exon 3 (of 833 bp) (Fig. 1C). No significant differences in the overall gene structure and organization between the bovine and human genes were observed.

POMC was mapped to the short arm of human chromosome 2 by Owerbach *et al.* (1981), and this mapping was refined by Zabel *et al.* (1983). In the light of the human genome sequencing, this location has been confirmed and it is apparent that POMC lies in a relatively uncluttered region between the DNA methyltransferase 3A gene (*DNMT3A*) and *EFR3B,* a gene of uncertain function (Fig. 1A,B). A number of *Alu* repeat elements have been identified within the *POMC* gene, the function of which is unclear. In the mouse genome, a second untranscribed *POMC* pseudogene has been identified, this being very similar to the third exon (Notake *et al.* 1983, Uhler *et al.* 1983).

A feature of the *POMC* gene that enabled its cloning was the high level of expression observed in pituitary and neurointermediate lobes in contrast to the near absence of expression in most other tissues. Understanding the mechanisms underlying this high degree of tissue specificity as well as the regulation of the gene by glucocorticoids was an immediately obvious and important question following the gene cloning. Access to the putative regulatory regions lying 5′ to the gene enabled these studies to begin. Jacques Drouin discusses this further in another review published in this issue (Drouin 2016); this will not be further considered here.

RNA studies

The cDNA cloning work described above had demonstrated that the pituitary and neurointermediate lobe mRNA

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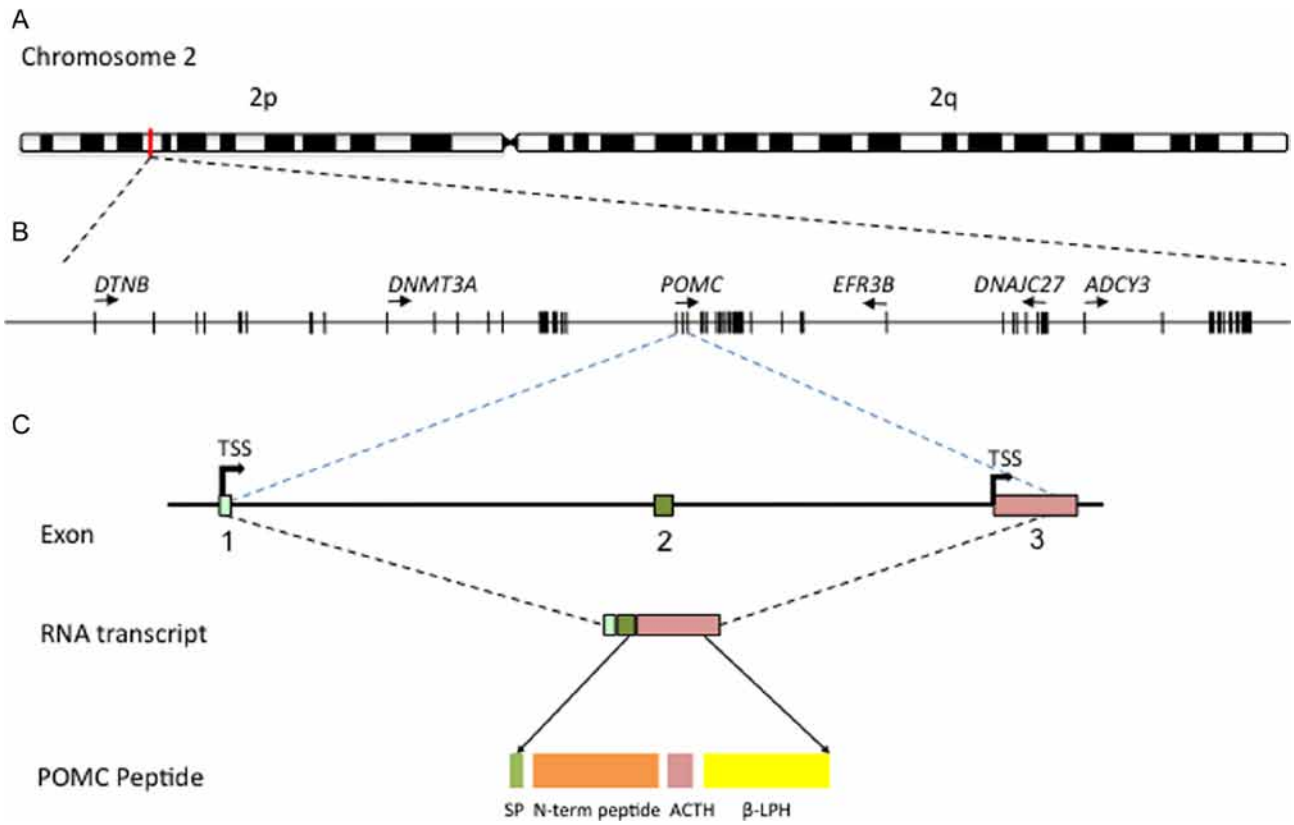

Figure 1
Anatomy of the *POMC* gene and its transcription and translation. (A) *POMC* is located on the short arm of human chromosome 2 in the region indicated by the vertical red line. This region is expanded in reverse orientation in (B) indicating those genes located in the vicinity of *POMC*. The direction of transcription for each of these genes is shown by the small horizontal arrows. Note the small size of *POMC* in comparison with other genes. An expanded view of POMC is shown in (C) indicating the three main exons and the two sites of transcription described in the text. The majority of transcripts start in exon 1 giving rise to the 1200 nt mRNA indicated. This RNA is translated from an initiator methionine in exon 2 to encode the POMC peptide.

transcript of the human gene consisted of 1072 nucleotides (nt) plus a poly-A tail of 100–200 nt, resulting in a mRNA transcript of around 1200 nt in total. Numerous studies using northern blotting techniques have confirmed this. Similar RNA sizes were reported in rat and porcine tissues by (Jeannotte *et al.* 1987), although they also observed a longer *POMC* mRNA species of around 1300 nt in hypothalamic mRNA. They demonstrated that this resulted from addition of a longer poly-A tail, a modification that has been postulated to confer greater stability on the transcript.

Although the pituitary (including the neurointermediate lobe in animals) is undoubtedly the major site of *POMC* gene expression, several reports have appeared of POMC peptide and mRNA expression in nonpituitary tissues including testis, ovary, placenta, lung, liver, thyroid and adrenal (Chen *et al.* 1984, 1986, Pintar *et al.* 1984, Lolait *et al.* 1986, DeBold *et al.* 1988*b*). Surprisingly, the mRNA in these tissues was approximately 800 nt: significantly shorter than that in the pituitary. Lacaze-Masmonteuil *et al.* (1987) and Jeannotte *et al.* (1987) showed that these transcripts only represented the third exon of the gene, and this would result in a peptide lacking the N-terminus of POMC including the signal sequence for peptide secretion. Indeed, Clark *et al.* (1990) demonstrated that while this transcript could be translated, the product was not normally secreted. The likely translational start site in this case would be methionine 53, which forms the fourth amino acid residue in the α-MSH/ACTH sequence. Lacaze-Masmonteuil *et al.* (1987) showed that these transcripts arose from a 'cryptic' GC-rich promoter region within intron 2 of the gene. No adequate evidence of any role for these transcripts has yet been put forward, and it is conceivable that this is a transcriptional 'accident'.

It is notable that recent data arising from the use of RNA sequencing shows low level of human *POMC* expression primarily, but not exclusively, derived from

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DOI: 10.1530/JME-15-0268

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exon 3 in many nonpituitary tissues including pancreas, stomach, appendix, kidney, testis, prostate, placenta, skin, adipose tissue, skeletal muscle, lymph node, brain, adrenal, and lung (http://www.proteinatlas.org/ENSG00000115138-POMC/tissue).

The clinical phenomenon of ectopic ACTH syndrome is a well-recognized, but uncommon syndrome in which a tumor arising from a nonpituitary tissue secretes ACTH, resulting in excessive cortisol secretion and Cushing's syndrome. Tsukada *et al.* (1981) had reported that a second longer *POMC* transcript coexisted alongside a typical 1200 nt species in a human ACTH secreting carcinoid tumor that gave rise to the ectopic ACTH syndrome. Similar observations were reported by others in a wide variety of ACTH-secreting extrapituitary tumors (Steenbergh *et al.* 1984, de Keyzer *et al.* 1985, Höppener *et al.* 1986, DeBold *et al.* 1988*a*, Clark *et al.* 1989). Various 5′ end mapping techniques suggest that although the majority of tumor *POMC* transcript originates at the conventional pituitary promoter, a few 'illegitimate' transcripts may arise from a more 5′ transcription start site. The underlying reasons for this observation have never really been fully elucidated.

The cloning of the *POMC* gene provided a unique insight into the structural nature of the peptide. However, as technology developed it became obvious that potentially great insights into the function of POMC and its derived peptides might be obtained by deleting this gene in the mouse. By chance, a naturally occurring *POMC* gene defect was identified in the human at approximately the same time. This review discusses the mouse models before turning to the human defects as the former provides a valuable model for the latter.

Pomc gene deletion in the mouse

The first attempts to manipulate the *Pomc* gene in mice were, perhaps surprisingly, not a complete gene deletion, but the introduction, via homologous recombination of a mutated *Pomc* allele, of a stop codon in the β-LPH sequence that would prevent any β-endorphin being produced (Rubinstein *et al.* 1996). Homozygous mice were healthy and exhibited normal behavior, but showed 10–15% weight gain after puberty when compared with wild-type animals. The mechanism for this is not clear. However, mice were shown to lose the opioid-dependent analgesia that could be induced by a swim test, but developed greater nonopioid analgesic mechanisms, presumably as a compensatory mechanism.

There have been two independent strategies used to target the murine *Pomc* gene, which have provided broadly similar results (Fig. 2). In view of the now well-recognized functions of MC1 receptors in the skin, MC4 receptors in the hypothalamus, and MC2 receptors in the adrenal cortex, it is no surprise that α-MSH and ACTH deficiency give rise to pigmentation abnormalities, obesity, and adrenal insufficiency, respectively, in homozygous animals. There are, however, some subtle and potentially instructive distinctions between these different experiments.

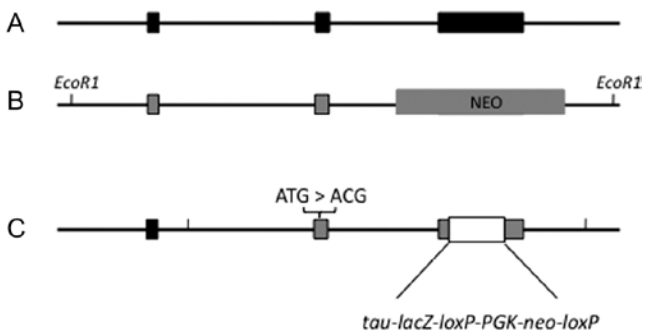

Figure 2
Mouse *Pomc* deletion strategies. (A) shows the normal 3 exon structure of *Pomc*. (B) Yaswen *et al.* (1999) used homologous recombination to replace a 9.5 kb EcoRI fragment of the gene with a *Pomc* fragment in which the neomycin resistance gene had replaced all of exon 3. The translation product would therefore include the first 44 codons encoded on exon 2 followed by the neomycin resistance gene. Grey boxes represent the inserted homologous sequences. (C) Challis *et al.* (2004) used a more complex strategy in which a targeting vector included a PCR generated fragment targeting the 5′ end of a 4.1 kb fragment including a mutation of the authentic ATG start codon, and an insertion within the exon 3 boundaries of a *tau-lacZ-PGK-neo* cassette (white box), in which the *PGK-neo* sequences were flanked by *loxP* sites for possible future use in creating conditional knockouts. Grey boxes represent the inserted homologous sequences.

Yaswen *et al.* (1999) used a neomycin resistance cassette to target the entire third exon of *Pomc* leading to loss of the majority of the *Pomc* coding sequence. Successful targeting was demonstrated by Southern blotting (showing the mutant allele) and absence of ACTH as measured by RIA in homozygous mice.

Challis *et al.* (2004) also targeted the third exon with a neomycin resistance sequence flanked by *Lox P* recombinase sites from a position 54 bp into the exon, so as to retain accurate splicing. The presence of the *Lox P* sites allowed the option for generation of conditional *Pomc* mutants in future studies. In addition, the exon 2 initiator methionine was mutated so that the N-terminal signal sequence and the 18 POMC amino acid residues encoded by this exon could not be produced – a theoretical disadvantage with the previous approach. Successful targeting was demonstrated by Southern blotting, northern blotting (to show absence of any POMC

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DOI: 10.1530/JME-15-0268

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Table 1 Summary of various mouse *POMC* gene deletion models and their key phenotypic characteristics.

Authors	Mouse	Weight	Adrenal	Comments
Rubinstein *et al.* (1996)	*PomcX*4*	10–15% greater than wt	Normal	Normal opioid analgesia/ Increased nonopioid analgesia
Yaswen *et al.* (1999)	*POMC tm2ute*	Obese, hyperphagic	Absent adrenal at 6 mo/Cort deficient	Some yellow pigmentation
Hochgeschwender *et al.* (2003)	*POMC tm2ute*	Obese, hyperphagic		Normal insulin/glucose uptake
Karpac *et al.* (2005)	*POMC tm2ute*	Obese, hyperphagic	Present at birth, then regress	
Challis *et al.* (2004)	*Pomc*$^{-/-}$	Obese, hyperphagic	Cort deficient	
Coll *et al.* (2004)	*Pomc*$^{-/-}$	Obese, hyperphagic	Hypotropic, absent zonal diff'n	ACTH × 10 days restores adrenal wt
Smart & Low (2003)	*POMC tm2ute* – C57 B6	Obese	Hypotropic	No pigmentation phenotype
Slominski (2005)	*POMC tm2ute* – C57 B6 *a/a*	Not stated	Not stated	Normal eumelanin production
Smart *et al.* (2006) Smart *et al.* (2007)	*Pomc*$^{-/-}$ *Tg*$^{/+}$	Obese +++, hyperphagic, Ins resistant	Normal/hyperplastic Pituitary tumors	Pituitary *Pomc* restored
Bumaschny *et al.* (2012)	*ArcPomc*$^{-/-}$*: CRE-ERT*	Normal/Obese	Not stated	Timed restoration reduces obesity
Lam *et al.* (2015)	*Lepr CRE/POMC Neo*	Normal	Not stated	POMC expressed in *Lepr* neurons only

transcript), and a two-site POMC immunoradiometric assay (to show absence of any POMC peptide).

Both groups bred these mice on a 129 Sv/Ev background initially, and both observed very similar reductions in the survival of homozygous mice to about 25% of that expected at term. It was found that addition of glucocorticoid to the maternal drinking water did not influence this lethality (Smart & Low 2003, Challis *et al.* 2004).

Smart and Low (2003) bred the Yaswen *Pomc* deletion (*Pomc tm2ute*) onto a C57 Bl/6 background providing a third model in which to study this phenotype. The advantage of this model is that this mouse strain is more inbred and hence more genetically homogeneous than the 129 mouse and it also has a predisposition to diet-induced obesity. As before, a very similar perinatal mortality among homozygous *Pomc*$^{-/-}$ mice was observed. These models are summarised in Table 1.

Obesity

All groups reported that surviving mice were indistinguishable from wild-type littermates until the second month of life when it became apparent that they were becoming obese. Substantial weight gain persisted and was accompanied by a small increase in body length. Furthermore, weight gain when placed on a high-fat diet was significantly greater than in wild-type animals. Dual energy X-ray absorptiometry scans reveal a greater fat and lean body mass in *Pomc*$^{-/-}$ animals. Mice were significantly hyperphagic and had reduced oxygen consumption, when corrected for body mass. Interestingly, heterozygous animals exhibit a very similar phenotype to wild types when raised on standard lab chow. On a high-fat diet, however, *Pomc*$^{+/-}$ mice do show significantly greater weight gain by 20 weeks. Biochemical analyses demonstrate that *Pomc*$^{-/-}$ mice are deficient in thyroxine, and, predictably, have substantially elevated plasma leptin. Circulating insulin is unchanged (Hochgeschwender *et al.* 2003). Mice with a C57 Bl/6 background had a similar obese phenotype (Smart & Low 2003).

These findings are entirely consistent with a deficiency of α-MSH resulting in absence of any agonist for the MC4 receptor, and recapitulate the observations made with MC4R-null mice (Huszar *et al.* 1997) and the *lethal yellow (Ay/a)* agouti mice (Bultman *et al.* 1992).

Availability of the *Pomc*$^{-/-}$ mice enabled an interesting series of experiments to be performed, in which the relative contributions of brain and pituitary *Pomc* to weight gain and pituitary–adrenal axis function could be examined. Smart *et al.* (2006, 2007) created a *Pomc* transgene on a *Pomc*$^{-/-}$ background, in which the transgene included pituitary-specific, but not brain-specific elements of its promoter. Resulting animals (*Pomc*$^{-/-}$ *Tg*$^{/+}$ mice) had *Pomc* expression restricted to the anterior pituitary, and had normal parameters of *Pomc* expression and ACTH production from the gland. However, despite this, animals develop adrenocortical hyperplasia with excess basal corticosterone, but reduced stress-induced corticosterone. Mice were shown to express excessive hypothalamic

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DOI: 10.1530/JME-15-0268

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corticotrophin-releasing hormone (CRH), implying that *Pomc* expression in the brain has a regulatory role on CRH expression. Furthermore, they are severely obese, hyperphagic, hyperleptinemic, and insulin resistant, reflecting the increased orexogenic effects of hypothalamic *Pomc* deficiency when normal or excessive glucocorticoid is available. Administration of glucocorticoid to *Pomc*$^{-/-}$ mice recapitulated this effect (Smart *et al.* 2007).

Bumaschny *et al.* (2012) conducted a clever and intriguing set of experiments in which a conditional rescue of *Pomc* deficiency in the hypothalamus was achieved by using a tamoxifen-controlled *Cre* excision of the neomycin resistance cassette in the arcuate nucleus. They showed convincing data of a reversal of the obesity, hyperphagia, and insulin resistance of these animals when *Pomc* was reintroduced. This effect lessened with age so that the effect was only partial when *Pomc* reintroduction occurred at 6 months. This same group subsequently used a leptin receptor-driven *Cre* recombinase to demonstrate that *Pomc* expression was only required in the leptin receptor-positive neurons of the hypothalamus for complete rescue of the obesity phenotype to be observed (Lam *et al.* 2015).

Pigmentation

POMC null mice on a 129 background exhibited yellow pigmentation, similar to, but not as prominent, as that seen in *Mc1r* deleted mice or agouti *Ay/a* mice (Yaswen *et al.* 1999). This observation is consistent with α-MSH deficiency, but the milder phenotype is compatible with the known constitutive activity of the MC1 receptor and the *Aw/Aw* genotype at the agouti locus. When bred onto a C57BL/6 background (*a/a* genotype), there was no discernable alteration in coat color, which may relate to the absence of agouti in this strain, or other factors (Smart & Low 2003). Slominski *et al.* (2005) bred this *Pomc*$^{-/-}$ strain onto 129/B6 mice with an *a/a* genotype and demonstrated absence of yellow pigmentation and normal eumelanin formation, consistent with the view that constitutive activity of the MC1R is sufficient to maintain eumelanin production in these animals.

Adrenal function

Deletion of *Pomc* and the consequent ACTH deficiency is likely to lead to adrenal insufficiency with undetectable corticosterone. Yaswen *et al.* (1999) confirmed corticosterone deficiency as well as aldosterone deficiency and reported that the adrenal glands themselves were impossible to find at 6 months of age. This suggested that ACTH or another POMC peptide was required for normal adrenocortical development. Heterozygous animals had near-normal corticosterone but, surprisingly, significantly reduced aldosterone. However, when the exon 3 deleted mice were crossed onto the C57 Bl/6 background, adrenals could be found, but were significantly smaller than the wild type (females: 15%; males: 30% of wild-type weight). Corticosterone was undetectable and remained so, even after receiving ACTH for 2 weeks.

As with the *tm2ute*, *Pomc*$^{-/-}$ mice corticosterone was undetectable and aldosterone was reduced in the exon 2/3 model described by Challis *et al.* (2004). Furthermore, it was possible to identify adrenals in all animals at 3 months of age, these being approximately 25% of the weight of wild-type adrenals. Microscopically, these adrenals had a distinguishable medulla and cortex, though further cortical zonation was not evident. Treatment of these mice for 10 days with subcutaneous depot ACTH resulted in a recovery of adrenal weight to normal levels and the appearance of normal cortical zonation. However, corticosterone was only minimally increased, and aldosterone was unchanged (Coll *et al.* 2004). Heterozygous animals had significantly reduced corticosterone levels when compared with wild types.

Further light was thrown on the adrenal discrepancies between these models by Karpac *et al.* (2005) using the *tm2ute* model. They demonstrated that at birth their *Pomc*$^{-/-}$ animals had adrenal glands that were indistinguishable from wild-type adrenals. Furthermore, animals born to *Pomc*$^{-/-}$ dams also had similar adrenal glands, arguing strongly that POMC peptides were not required for antenatal adrenal development. Soon after birth, however, adrenals failed to grow in knockout animals, and by 6 months of age were undetectable – as was reported by Yaswen *et al.* (1999). Furthermore, transplantation of *Pomc*$^{-/-}$ adrenals into wild-type animals permits full recovery of the gland. This suggests that ACTH, or possibly another POMC peptide is required for adrenal gland maintenance, but not for its development.

Although POMC(1-28) has been shown to be an adrenal mitogen in a number of *in vivo* and *in vitro* experimental studies in rats (Estivariz *et al.* 1982, 1988) and in human NCI-H295 and mouse Y-1 adrenal tumor cell lines and primary cultures of bovine adrenocortical cells (Fassnacht *et al.* 2003). Coll *et al.* (2006) showed that administration of the same peptide to *Pomc*$^{-/-}$ mice for 10 days was without effect on adrenal weight or morphology, and when given with ACTH the effects did not differ from those with ACTH alone.

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Table 2 Human homozygous and compound heterozygous *POMC* gene defects.

Authors	DNA seq change	Exon	Protein defect	Phenotype
Krude *et al.* (1998)	G12119T/C12240insC	3	E131X/F144fs	AI, obesity, red hair
	C8908A	2	Alt cistron	AI, obesity, red hair
Krude *et al.* (2003)	C8908A	2	Alt cistron	AI, obesity, red hair
	A11957T/12102delG	3	K51X/G99fs	AI, obesity, red hair
	C8908A/12208insGG	2+3	Alt cistron/E134fs	AI, obesity, red hair
Farooqi *et al.* (2006)	12012delC	3	P69L	AI, obesity, hyperphagia
Clément *et al.* (2008)	12028insC	3	P74fs	AI, obesity, GH def, TSH def
Mendiratta *et al.* (2011)	C12037A	3	pY77X	AI, hyperphagia, obesity
Samuels *et al.* (2013)	C8908A/C12241T	2+3	Alt cistron/R145C	AI, obesity, red hair, elevated ACTH
	C12241T	3	R145C	

Where only one variant is shown, the patient was homozygous for that variant. Alt cistron, alternative cistron; AI, adrenal insufficiency. DNA sequence numbering is based on NCBI Reference Sequence: NG_008997.1 and protein sequence numbering is based on NCBI Reference Sequence: XP_011531219.1.

An interesting series of observations that emerged later was that *Pomc*$^{-/-}$ and *Pomc*$^{+/-}$ mice developed pituitary tumors arising from melanotroph or corticotroph cells in either the neurointermediate or anterior lobes (respectively) from 12 months of age (Smart *et al.* 2007). These tumors showed reticulin breakdown and increased numbers of mitotic figures typical of adenoma formation. Tumor formation seems to correlate well with increased hypothalamic *CRH* mRNA in these animals, although a contribution from absent glucocorticoid feedback may also be important.

Human *POMC* gene defects

The first description of a human *POMC* defect in fact preceded the publication of the *Pomc* deleted mouse, and the clinical features accurately anticipated those that were later described in the mouse.

Krude *et al.* (1998) reasoned that a syndrome combining severe childhood obesity and adrenal insufficiency from birth perhaps together with pigmentation abnormalities would be the most likely phenotype resulting from a *POMC* mutation. They investigated this possibility in two unrelated children with precisely this syndrome and identified mutations in *POMC*. In their first patient, whose older brother had died at 7 months of age, adrenal hypoplasia had been identified and glucocorticoid replacement was started during the neonatal period. Excessive weight gain became apparent from about 3 months of age as was red hair and a pale skin. *POMC* sequencing identified a compound heterozygous mutation resulting in the introduction of a stop codon at position 131 in one allele, terminating translation in the joining peptide, and a frameshift in residue 144 followed by a nonsense sequence and a premature termination in the other allele, terminating the normal POMC sequence after the seventh residue of the α-MSH/ACTH sequence.

Their second patient, identified at age 5, had an almost identical syndrome. *POMC* sequencing revealed a novel type of homozygous mutation that created a new initiator methionine just upstream of the conventional site, which resulted in a short, out-of-frame translation product that prevents translation from the correct site. This would result in complete absence of any POMC peptide.

As this report, a number of other mutations within the POMC coding region have been identified in children with a very similar severe phenotype (OMIM #609734). These are summarized in Table 2 and in Fig. 3. It is interesting that the red hair phenotype is not constant and was absent in cases described by Farooqi *et al.* (2006), Clément *et al.* (2008), and Mendiratta *et al.* (2011). Furthermore, one

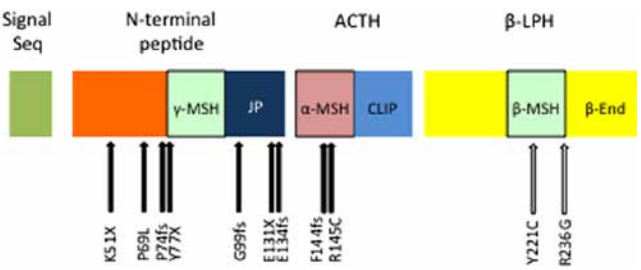

Figure 3
POMC mutations. Representation on a cartoon of the POMC peptide of the homozygous or compound heterozygous coding region variants that have been associated with the *POMC* gene deficiency syndrome (OMIM #609734) in the filled black arrows. Unfilled arrows indicate the location of the heterozygous defects that have been associated with defective β-MSH processing or function. The boxed regions represent the three MSH sequences in the peptide. JP, joining peptide; CLIP, corticotrophin-like intermediate peptide; β-end, β-endorphin.

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DOI: 10.1530/JME-15-0268

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case developed growth hormone deficiency and thyroid-stimulating hormone deficiency at puberty (Clément *et al.* 2008).

An extremely interesting variation on this phenotype was described by Samuels *et al.* (2013) in two unrelated patients who presented with a typical syndrome of adrenal insufficiency, obesity, and red hair. The major distinction of these cases was that they had markedly elevated circulating ACTH. Sequencing revealed that both cases were compound heterozygous (case 1) or homozygous (case 2) for a missense mutation of the eighth residue of the ACTH peptide such that the invariant arginine was converted to cysteine. Since this residue is central to the characteristic His-Phe-Arg-Trp sequence of α-MSH and ACTH, this mutation produces biologically inactive, but immunologically detectable ACTH and α-MSH peptides.

Heterozygous effects

It was apparent from some of the earliest studies described above that heterozygous family members had a significantly greater frequency of obesity than expected. Thus, for example, in one study there were 12 heterozygous and 7 normal homozygous relatives. BMI was significantly greater in the heterozygotes than the wild types (Farooqi *et al.* 2006). This finding implied that single allele defects of *POMC* could contribute toward development of an obese phenotype. This has prompted a series of studies in which *POMC* was sequenced in cohorts of obese individuals. As a result, a series of heterozygous nonsynonymous variants of the gene were identified (Table 3). Although in most of these cases a functional analysis of the effect of the variant was not conducted, two cases are particularly worth considering.

Challis *et al.* (2002) reported two patients among their cohort in whom the β-MSH–β-endorphin Lys-Arg cleavage site was disrupted, resulting in production of a β-MSH–β-endorphin fusion protein that they demonstrated *in vitro* was less efficient at activating the MC4 receptor by one order of magnitude. Whether this would be the case *in vivo* remains uncertain as there is little evidence for cleavage of the N-terminal Lys-Lys site flanking the β-MSH sequence *in vivo* in the human (see Lowry, this issue). Whether it were the case that β-MSH was a functional peptide in the hypothalamus, it would be hard to see why homozygous mutations affecting α-MSH, for example, Samuels *et al.* (2011), would have such a potent effect on appetite.

In a second study, Lee *et al.* (2006) reported five severely obese unrelated patients with heterozygous Y221C missense mutations. Tyrosine 221 is the sixth residue of the putative β-MSH peptide, and they demonstrated *in vitro* that the synthetic mutant peptide was less active at the MC4 receptor than the wild type. An alternative and potentially more damaging pathogenic mechanism for this mutation, however, is that it is highly likely to lead to a second disulfide bridge (human POMC initially exists as a dimer due to a disulfide bridge arising from the single cysteine residue in the joining peptide) resulting in a more stable dimer in the secretory pathway possibly culminating in proteosomal degradation of POMC, which in turn would significantly reduce overall POMC peptide secretory capacity by a dominant negative effect (Phil Lowry, personal communication).

Given the evidence outlined above that heterozygous POMC variants may be associated with obesity, one might predict that POMC was a significant quantitative trait locus (QTL) for obesity in genome-wide association studies. In fact, this does not appear to be the case in most studies. For example, in one recent report *POMC* was not among 100 QTLs that were identified or confirmed in a population of over 320,000 individuals (Winkler *et al.* 2015). Voisin *et al.* (2015) have reported that methylation of a single nucleotide polymorphism within the POMC promoter region was among methylation site variants from 28 genes associated with obesity.

Table 3 Human heterozygous *POMC* gene variants.

Authors	Population screened	Variants identified	Comments
Hinney *et al.* (1998)	96 obese children	p99 ins SSCSSCp202 ins RA, pE206X, & pE214G	Three variants in one individual
Miraglia del Giudice (2001)	87 obese Italian children	pS7T, pS9L, pR236G	
Challis *et al.* (2002)	262 obese Caucasian	pR236G (2)	Segregates with obesity
Santoro *et al.* (2004)	380 obese Italian children	p99 ins SSG	Associates with fasting insulin
Buono (2005)	196 obese Italians	p231L, pE244X, pR236G	1.5% of obese cohort
Lee *et al.* (2006)	538 obese Caucasian	pY221C	Role for β-MSH implied
Dubern *et al.* (2008)	322 obese French children	pF144L	Part of α-MSH HFRW core sequence
Creemers *et al.* (2008)	500 obese Caucasian	pC28F, pL37F	Impaired POMC processing

Protein sequence numbering is based on NCBI Reference Sequence: XP_011531219.1.

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Although it has not been studied to the same extent, there is currently no evidence that adrenal function is similarly affected by heterozygosity for *POMC* mutations.

Summary

The contribution made as a result of the isolation of the *POMC* gene cannot be understated. As has been reviewed, its original isolation and cloning was the result of some brilliant science in the earliest days of the molecular biological revolution. This discovery provided incontrovertible evidence that the common precursor hypothesis for ACTH and β-LPH was right, and this probably inspired many young researchers (including the author) of the enormous potential of molecular endocrinology research.

The physiological roles of the POMC peptides, especially α-MSH and ACTH, have been carefully dissected by many researchers, but the development of animal models in which POMC was deleted or cleverly manipulated provided a much clearer understanding of these roles. This was also true of the almost simultaneous discoveries of *POMC* gene variants in human disease and its particularly critical role in obesity.

Declaration of interest
The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

Funding
This work did not receive any specific grant from any funding agency in the public, commercial, or not-for-profit sector.

Acknowledgements
The author would like to acknowledge helpful discussions with L Metherell and Professor P Lowry in preparing this article.

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Received in final form 22 November 2015
Accepted 4 December 2015
Accepted Preprint published online 7 December 2015

http://jme.endocrinology-journals.org
DOI: 10.1530/JME-15-0268

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