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 adrenocorticotropic 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

Introduction

The concept that one precursor peptide, adrenocorticotropic hormone-β-lipotropin (ACTH-β-LPH), could contain more than 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...
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 32P-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 Overbach 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...
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. 1988b). Surprisingly, the mRNA in these tissues was approximately 800 nt: significantly shorter than that in the pituitary. Lacaze-Masmonteil 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-Masmonteil 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

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.
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 tumor that gave rise to the ectopic ACTH syndrome. Similar 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 gene POMC was mutated in mice that would prevent any 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) also targeted the 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 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. 

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.

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.

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 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.

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
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 Pmc−/− mice was observed. These models are summarised in Table 1.

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

<table>
<thead>
<tr>
<th>Authors</th>
<th>Mouse</th>
<th>Weight</th>
<th>Adrenal</th>
<th>Comments</th>
</tr>
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<tbody>
<tr>
<td>Rubinstein et al. (1996)</td>
<td>PomcX*4</td>
<td>10–15% greater than wt</td>
<td>Normal</td>
<td>Normal opioid analgesia/ Increased nonopioid analgesia Some yellow pigmentation</td>
</tr>
<tr>
<td>Yaswen et al. (1999)</td>
<td>POMC tm2ute</td>
<td>Obese, hyperphagic</td>
<td>Absent adrenal at 6 mol/Cort deficient</td>
<td>Normal insulin/glucose uptake</td>
</tr>
<tr>
<td>Hochgeschwender et al. (2003)</td>
<td>POMC tm2ute</td>
<td>Obese, hyperphagic</td>
<td>Present at birth, then regress Cort deficient Hypotrophic, absent zonal diff'n</td>
<td>ACTH x 10 days restores adrenal wt No pigmentation phenotype Normal eumelanin production Pituitary Pmc restored</td>
</tr>
<tr>
<td>Karpac et al. (2005)</td>
<td>POMC tm2ute</td>
<td>Obese, hyperphagic</td>
<td>Hypotrophic</td>
<td>Normal/ hyperplastic Pituitary Pmc restored</td>
</tr>
<tr>
<td>Challis et al. (2004)</td>
<td>Pmc−/−</td>
<td>Obese, hyperphagic</td>
<td>Not stated</td>
<td>Normal weight</td>
</tr>
<tr>
<td>Coll et al. (2004)</td>
<td>Pmc−/−</td>
<td>Obese, hyperphagic</td>
<td>Hypotrophic</td>
<td>Normal weight</td>
</tr>
<tr>
<td>Smart &amp; Low (2003)</td>
<td>POMC tm2ute – C57 B6</td>
<td>Obese</td>
<td>Hypotrophic</td>
<td>ACTH x 10 days restores adrenal wt No pigmentation phenotype Normal eumelanin production Pituitary Pmc restored</td>
</tr>
<tr>
<td>Slominski (2005)</td>
<td>POMC tm2ute – C57 B6</td>
<td>Not stated</td>
<td>Not stated</td>
<td>Normal weight</td>
</tr>
<tr>
<td>Smart et al. (2006)</td>
<td>Pmc−/− Tg+</td>
<td>Obese +++, hyperphagic, Ins resistant</td>
<td>Normal/Obese</td>
<td>Pituitary Pmc restored</td>
</tr>
<tr>
<td>Bumaschny et al. (2012)</td>
<td>Lepr CRE/ POMC Neo</td>
<td>Normal</td>
<td>Not stated</td>
<td>Normal weight</td>
</tr>
</tbody>
</table>

**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 Pmc−/− 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, Pmc−/− mice do show significantly greater weight gain by 20 weeks. Biochemical analyses demonstrate that Pmc−/− 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 Pmc−/− mice enabled an interesting series of experiments to be performed, in which the relative contributions of brain and pituitary Pmc to weight gain and pituitary–adrenal axis function could be examined. Smart et al. (2006, 2007) created a Pmc transgene on a Pmc−/− background, in which the transgene included pituitary-specific, but not brain-specific elements of its promoter. Resulting animals (Pmc−/− Tg+ mice) had Pmc expression restricted to the anterior pituitary, and had normal parameters of Pmc 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 hypotalamic...
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 orexigenic 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 (Estivartiz 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.
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 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

<table>
<thead>
<tr>
<th>Authors</th>
<th>DNA seq change</th>
<th>Exon</th>
<th>Protein defect</th>
<th>Phenotype</th>
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<td>G12119T/C12240insC</td>
<td>3</td>
<td>E131X/F144fs</td>
<td>AI, obesity, red hair</td>
</tr>
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<td>Krude et al.</td>
<td>C8908A</td>
<td>2</td>
<td>Alt cistron</td>
<td>AI, obesity, red hair</td>
</tr>
<tr>
<td></td>
<td>C8908A</td>
<td>2</td>
<td>Alt cistron</td>
<td>AI, obesity, red hair</td>
</tr>
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<td></td>
<td>A11957T/12102delG</td>
<td>3</td>
<td>K51X/G99fs</td>
<td>AI, obesity, red hair</td>
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<tr>
<td></td>
<td>C8908A/12208insGG</td>
<td>2+3</td>
<td>Alt cistron/E134fs</td>
<td>AI, obesity, red hair</td>
</tr>
<tr>
<td>Farooqi et al.</td>
<td>12012delC</td>
<td>3</td>
<td>P69L</td>
<td>AI, obesity, hyperphagia</td>
</tr>
<tr>
<td>Clément et al.</td>
<td>12028insC</td>
<td>3</td>
<td>P74fs</td>
<td>AI, obesity, GH def, TSH def</td>
</tr>
<tr>
<td>Mendiratta et</td>
<td>C12037A</td>
<td>3</td>
<td>pY77X</td>
<td>AI, hyperphagia, obesity</td>
</tr>
<tr>
<td>Samuels et al.</td>
<td>C8908A/C12241T</td>
<td>2+3</td>
<td>Alt cistron/R145C</td>
<td>AI, obesity, red hair, elevated ACTH</td>
</tr>
<tr>
<td></td>
<td>C12241T</td>
<td>3</td>
<td>R145C</td>
<td></td>
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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.
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 heterozygous and normal homozygous relatives. 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.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Population screened</th>
<th>Variants identified</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hinney et al. (1998)</td>
<td>96 obese children</td>
<td>p99 ins SSCSSCp202 ins RA</td>
<td>Three variants in individual</td>
</tr>
<tr>
<td>Challis et al. (2002)</td>
<td>262 obese Caucasian</td>
<td>pR236G (2)</td>
<td>Associates with fasting insulin</td>
</tr>
<tr>
<td>Santoro et al. (2004)</td>
<td>380 obese Italian children</td>
<td>p99 ins SSG</td>
<td>1.5% of obese cohort</td>
</tr>
<tr>
<td>Buono (2005)</td>
<td>196 obese Italians</td>
<td>p231L, pE244X, pR236G</td>
<td>Role for β-MSH implied</td>
</tr>
<tr>
<td>Lee et al. (2006)</td>
<td>538 obese Caucasian</td>
<td>pY221C</td>
<td>Part of α-MSH HFRW core sequence</td>
</tr>
<tr>
<td>Dubern et al. (2008)</td>
<td>322 obese French children</td>
<td>pF144L</td>
<td>Impaired POMC processing</td>
</tr>
<tr>
<td>Creemers et al. (2008)</td>
<td>500 obese Caucasian</td>
<td>pC28F, pL37F</td>
<td></td>
</tr>
</tbody>
</table>

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

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