

WestminsterResearch

<http://www.westminster.ac.uk/westminsterresearch>

**Emerging roles of melanocortin receptor accessory proteins
(MRAP and MRAP2) in physiology and pathophysiology**

Berruien, N. and Smith, C.L.

NOTICE: this is the authors' version of a work that was accepted for publication in Gene. Changes resulting from the publishing process, such as peer review, editing, corrections, structural formatting, and other quality control mechanisms may not be reflected in this document. Changes may have been made to this work since it was submitted for publication. A definitive version was subsequently published in Gene, Volume 757, 5 October 2020, 144949 757, 2020.

The final definitive version in Gene is available online at:

<https://dx.doi.org/10.1016/j.gene.2020.144949>

© 2020. This manuscript version is made available under the CC-BY-NC-ND 4.0 license

<https://creativecommons.org/licenses/by-nc-nd/4.0/>

The WestminsterResearch online digital archive at the University of Westminster aims to make the research output of the University available to a wider audience. Copyright and Moral Rights remain with the authors and/or copyright owners.

Emerging roles of melanocortin receptor accessory proteins (MRAP and MRAP2) in physiology and pathophysiology.

Abstract:

Melanocortin-2 receptor accessory protein (MRAP) has an unusual dual topology and influences the expression, localisation, signalling and internalisation of the melanocortin receptor 2 (MC2); the adrenocorticotrophic hormone (ACTH) receptor. Mutations in MRAP are associated with familial glucocorticoid deficiency type-2 and evidence is emerging of the importance of MRAP in adrenal development and ACTH signalling. Human MRAP has two functional splice variants: MRAP- α and MRAP- β , unlike MRAP- β , MRAP- α has little expression in brain but is highly expressed in ovary. MRAP2, identified through whole human genome sequence analysis, has approximately 40% sequence homology to MRAP. MRAP2 facilitates MC2 localisation to the cell surface but not ACTH signalling. MRAP and MRAP2 have been found to regulate the surface expression and signalling of all melanocortin receptors (MC1-5). Additionally, MRAP2 moderates the signalling of the G-protein coupled receptors (GPCRs): orexin, prokineticin and GHSR1a; the ghrelin receptor.

Whilst MRAP appears to be mainly involved in glucocorticoid synthesis, an important role is emerging for MRAP2 in regulating appetite and energy homeostasis. Transgenic models indicate the importance of MRAP in adrenal gland formation. Like MC3R and MC4R knockout mice, MRAP2 knockout mice have an obese phenotype. In vitro studies indicate that MRAP2 enhances the MC3 and MC4 response to the agonist α MSH, which, like ACTH, is produced through precursor polypeptide proopiomelanocortin (POMC) cleavage. Analysis of cohorts of individuals with obesity have revealed several MRAP2 genetic variants with loss of function mutations which are causative of monogenic hyperphagic obesity with hyperglycaemia and hypertension. MRAP2 may also be associated with female infertility. This review summarises current knowledge of MRAP and MRAP2, their influence on GPCR signalling, and focusses on pathophysiology, particularly familial glucocorticoid deficiency type-2 and obesity.

Keywords:

MRAP; MRAP2; MC2; MC1R; MC2R; MC3R; MC4R; MC5R; melanocortin; ACTH; dual topology; ghrelin; alpha-MSH; adrenal gland; glucocorticoid; female infertility; familial glucocorticoid deficiency; melanocortin 2; receptor accessory protein; melanocortin receptor; MC; obesity

Introduction

Genetic variants of melanocortin-2 receptor accessory protein (*MRAP*) are associated with severe glucocorticoid deficiency (Metherell, et al., 2005). Adrenocorticotrophic hormone (ACTH) is the endogenous agonist for the melanocortin receptor 2 (MC₂), a class A G-protein coupled receptor. MRAP is a small transmembrane protein which promotes the assembly of melanocortin receptor 2 in the endoplasmic reticulum and

45 stabilises MC₂ in the plasma membrane. MRAP influences the binding of ACTH to
46 MC₂ and following agonist stimulation, MC₂ via Gs protein, activates adenylate cyclase
47 generating cyclic adenosine monophosphate (cAMP).

48

49 Effective ACTH signalling is vital to produce steroids including glucocorticoid, hence
50 genetic variants of *MRAP* are linked to familial glucocorticoid deficiency. *MRAP*
51 expression is high in the brain and the adrenal gland and MRAP has been
52 demonstrated to affect the differentiation of progenitor cells within the adrenal gland
53 (Novoselova, et al., 2018).

54 *MRAP2* shares approximately 40% sequence homology with *MRAP* but unlike MRAP,
55 which facilitates MC₂ localisation and signalling; MRAP2 has less receptor fidelity,
56 influencing the signalling of melanocortin receptors (MC₁₋₅), as well as orexin receptor,
57 ghrelin receptor and growth hormone secretagogue receptor 1a (GHSR-1a) (Srisai, et
58 al., 2017). MC₃ and MC₄ both have important roles in appetite control with deficiencies
59 linked to obesity (Chen, et al., 2000). The MC₄ agonist setmelanotide is currently in
60 phase 3 clinical trials for rare genetic disorders of obesity caused by mutations in
61 proopiomelanocortin (*POMC*) (Kühnen, et al., 2016) and leptin receptor (*LEPR*)
62 (Clément, et al., 2018). Evidence from *in vitro* analyses, MRAP2 transgenic animals
63 and *MRAP2* variants in populations with metabolic syndrome (Baron, et al., 2019),
64 indicate that MRAP2 has an important role regulating appetite control, with *MRAP2*
65 genetic variants causing obesity.

66

67 This article will review the structures of melanocortin receptor accessory proteins,
68 physiological roles, and emerging evidence about the *MRAP* and *MRAP2* genetic
69 variants which shed light upon pathophysiological conditions.

70

71

72 **Protein structure**

73 MRAP is a hydrophobic single pass transmembrane protein which is encoded for by
74 a single gene located on chromosome 21q22.11 (Xu, et al., 2002) composed of 6
75 exons. There are two functional MRAP splice variants: MRAP- α derived from exon 1-
76 5 which is 172 amino acids (19 kDa) and MRAP- β derived from exon 1-4, 6, which is
77 102 amino acids (14.1 kDa). These splice variants have conserved N-terminal
78 homology but differ at the C-terminus (Xu, et al., 2002; Metherell, et al., 2005)

79 When first described, it was predicted that the MRAP dimer would orientate with
80 intracellular N-terminus and extracellular C-terminus (Metherell, et al., 2005).
81 However, through epitope immunoprecipitation and live cell imaging studies the
82 quaternary structure of the MRAP was found to form a stable anti-parallel homodimer
83 with N-termini orientating both intracellularly and extracellularly, which is still thought
84 to be unique in eukaryotes (Sebag and Hinkle, 2007).

85 MRAP is partially glycosylated and this is dependent upon the Asn-X-Ser/Thr motif
86 facing the luminal surface of the endoplasmic reticulum (Sebag and Hinkle, 2007),

87 however, the creation of functional MRAP glycosylation mutants in this study
88 determined that glycosylation was not necessary for dual topology.

89

90 A further series of mutational studies identified the N-terminal 31-37 sequence
91 (LKANKHS) adjacent to the transmembrane region to be essential for this dual
92 topology (Sebag, and Hinkle, 2009). Using bimolecular fluorescence
93 complementation, Sebag and Hinkle demonstrated that this dual topology occurs in
94 the endoplasmic reticulum; once formed this stable orientation is retained when the
95 protein is expressed on the plasma membrane (Maben, et al., 2016).

96

97 The N-terminus and the transmembrane domains of MRAP are conserved between
98 species however, the carboxyl region has greater variation (Agulleiro, et al., 2010).
99 The 18-21 LDYI sequence is unique to the MRAP isoform and is required for the
100 binding of ACTH to melanocortin receptor 2 (Sebag, and Hinkle, 2009) but MRAP C-
101 terminus truncation experiments suggested this domain has less impact on ACTH
102 signalling (Sebag, and Hinkle, 2009). However, truncated MRAP (with the removal of
103 the C-terminal domain), expressed in HEK293/FRT cells, revealed diminished MC₂
104 surface expression and cAMP production compared to full length MRAP; suggesting
105 that the C-terminus does modulate MC₂ cAMP generation in response to ACTH (Roy,
106 et al., 2012). In addition serine and threonine residues in the C-terminus are necessary
107 for MC₂ internalisation and recycling (Roy, et al., 2011). The exact residues necessary
108 for MRAPs to interact with GPCRs have not been fully characterised; however, co-
109 precipitation studies with MC₂ suggest that the MRAP transmembrane domain is
110 necessary (Webb, et al., 2009).

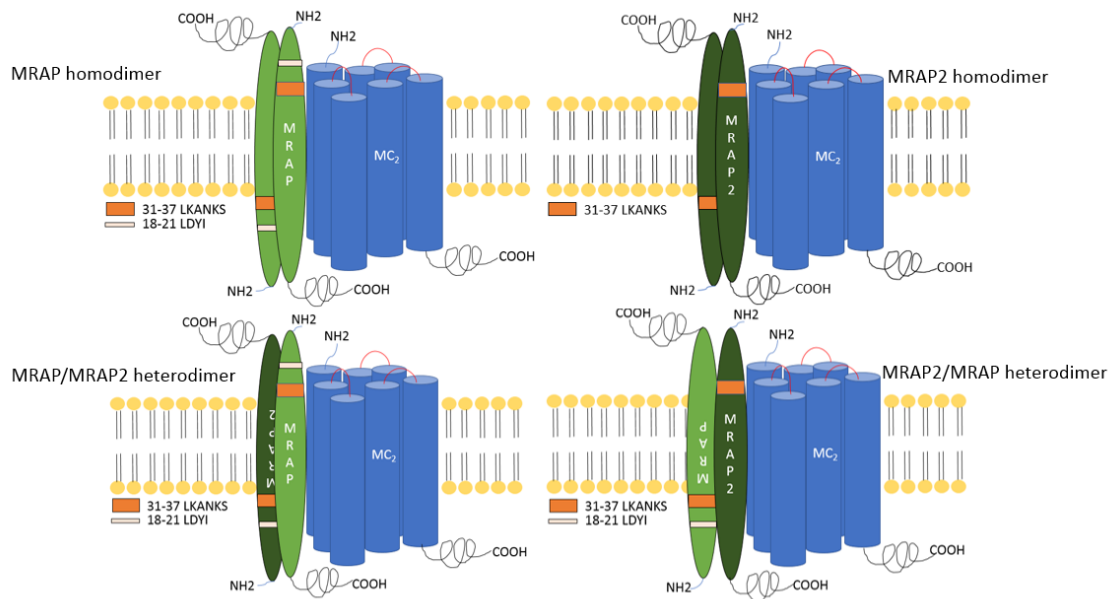
111

112 **Discovery, Structure and Characteristics of MRAP2**

113 As a paralog to *MRAP*, *MRAP2* was identified in the human genome after sequence
114 analysis. The *MRAP2* gene is located at 6q14.3 and encodes for a 205 amino acid
115 protein, the sequence alignment of *MRAP2* indicates approximately 40% homology
116 with *MRAP*. The greatest sequence conservation lies at the N-terminal and within the
117 transmembrane domain, whilst the carboxyl terminal differs greatly between these two
118 proteins (Chan, et al., 2009).

119 MRAP2 is a transmembrane protein that traverses the membrane once and as with
120 the MRAP, MRAP2 could be found both on the surface membrane and subcellularly
121 on the luminal surface of the endoplasmic reticulum (Sebag, and Hinkle, 2010).
122 MRAP2 shares the dual orientation of the carboxyl and amino terminal forming an
123 antiparallel homodimer (Figure 1). Interestingly, MRAP2 has been shown to form a
124 heterodimer with the MRAP (Figure 1); a yellow fluorescent protein in bimolecular
125 complementation studies revealed an antiparallel orientation of the MRAP-MRAP2
126 heterodimer (Sebag, and Hinkle, 2010).

127



128

129

130 Figure 1: Dual topology of MRAP homodimer, MRAP2 homodimer and MRAP/MRAP2
 131 heterodimer; the green transmembrane domain represents the structure and the dual
 132 topology with the amino (NH₂) / carboxyl (COOH) both being located extracellularly
 133 and amino (NH₂) / carboxyl (COOH) termini also intracellularly. This dual topology
 134 allows for homodimer (MRAP/MRAP; MRAP2/MRAP2) or heterodimer
 135 (MRAP/MRAP2; MRAP2/MRAP) formation. The LKANKS₃₁₋₃₇ (orange shading)
 136 immediately before the transmembrane region is required for dual topology, the
 137 LDYI₁₈₋₂₁ (yellow shading) is found only in MRAP and is required for ACTH binding to
 138 MC₂.

139

140

141 MRAP gene regulation

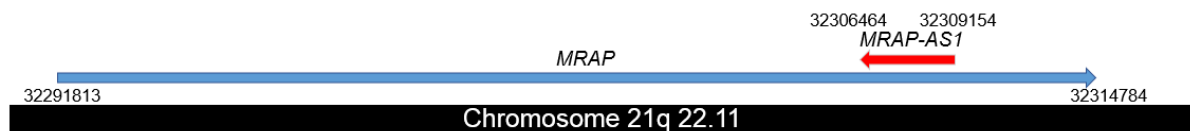
142

143 *MRAP* gene expression is upregulated by lipopolysaccharide (Gibbison, et al., 2015),
 144 ACTH (Liu, et al., 2013) and in adipocytes by PPAR γ (peroxisome proliferator-
 145 activated receptor gamma) (Kim, et al., 2013). Diurnal *Mrap* expression has been
 146 observed in rats, with *Mrap* mRNA correlating with the expression of circadian
 147 regulating genes (*per1*, *per2* and *CLOCK*) (Spiga, et al., 2020). Since the *MRAP*
 148 mRNA half-life is shorter than *MC2R*, Clark and Chan, (2019) have proposed a
 149 model where MRAP may facilitate the transport of MC₂ to the plasma membrane
 150 without necessitating *de novo* MRAP/MC₂R expression.

151

152 Gnomon automated computational analysis of the 21q22.11 region of the
 153 GRCh38.p13 (Souvorov, et al., 2010) has predicted the presence of a novel *MRAP*
 154 antisense RNA 1 (*MRAP-AS1*; XR_937664.1). Whether this non-coding RNA has a
 155 biological function and can regulate *MRAP* remains to be determined.

156



157

158 Figure 2: Predicted non-coding RNA, *MRAP-AS1* (XR_937664.1, shown in red) on
159 chromosome 21q22.11 in relation to *MRAP* (blue)

160

161

162 The evolution of MRAPs

163

164 Phylogenetic studies have revealed the existence of the *MRAPs* orthologues in
165 different piscine species: zebrafish, tetrapod and some bony fish subspecies
166 (Valsalan, et al., 2012). Interestingly, whilst *MRAP2* was found in the sea lamprey and
167 cartilaginous fish, *MRAP* was absent which lead to a theory that *MRAP2* gene
168 duplication may have given rise to *MRAP* (Västermark, and Schiöth, 2011). However,
169 cDNA blast analysis from the elephant shark genome project located the *MRAP*
170 sequence (Venkatesh, et al., 2014) discrediting an *MRAP2* ancestral role. Considering
171 all these findings, the origin of the *MRAPs* genes is thought to be as a result of R2
172 genome duplication events according to the evolution theory (Ohno et al., 1968); a
173 comprehensive review of melanocortin receptor evolution has been presented by
174 Dores (Dores, et al., 2016).

175

176 The presence of both *MRAPs* has been described in most mammals and chicken
177 (Valsalan et al., 2012), the dual topology feature of *MRAP* and *MRAP2* was conserved
178 through the appearance of the motifs LKANKH (*MRAP*) and LKAHKY (*MRAP2*) in the
179 orthologs of mice, chicken and zebrafish. Additionally, sequence alignment studies of
180 all lineages revealed the *MRAP* sequence to be shorter than the *MRAP2* sequence,
181 with the transmembrane domain being well conserved amongst all species examined.

182

183 Distribution of the MRAPs

184

185 In humans reverse transcription PCR (RT-PCR) indicated that both isoforms are
186 present at high levels in the adrenals, testis and breasts; with low expression in in the
187 digestive system, heart, fat and skin (Metherell, et al., 2005). In humans, *MRAP- α* and
188 *MRAP- β* were found expressed in the adipose tissue, breast, testis and adrenal cortex
189 (see table 1).

190 Examining the “Human Protein Atlas (HPA)- RNA-seq” data of 95 human samples
191 reveals a wide distribution of *MRAP*, with the highest expression levels observed in
192 the adipose tissue and the adrenal gland. This is followed by the skin and the male
193 reproductive organs whilst brain, heart, ovary and the kidney had a lower expression
194 (Fagerberg, et al., 2014). Table 1 lists human tissues expressing the *MRAP- α* and
195 *MRAP- β* . Along with the adrenal gland expression, *MRAP2* was expressed in different
196 parts of the brain at high levels, as shown in the HPA-RNA-seq data (Fagerberg, et

197 al., 2014). *MRAP2* was also found to be highly expressed in the female and male
198 reproductive systems and stomach whilst endocrine glands, liver and peripheral blood
199 had the lowest *MRAP2* expression (Fagerberg, et al., 2014).

200

201 As discussed above, the *MRAPs* are present in other species, including chicken, fish
202 and mice (Zhang et al., 2017; Agulleiro et al., 2010; Asai et al., 2013; Valsalen et al
203 2012). While the *MRAP* was widely distributed in the tissues of those species, the
204 *MRAP2* expression is mainly localised to the brain.

205

206

207
208
209

Table 1: Expression of *MRAP-α*, *MRAP-β* and *MRAP2* in human tissues. +++: high expression; +: low expression.

Tissue	<i>MRAP-α</i>	<i>MRAP-β</i>	<i>MRAP2</i>	Reference
Adrenal gland	+++	+++	+++	Metherell et al., 2005 Chan et al., 2009
Testis	+++	+++	+	Metherell et al., 2005
Adipose	+	+	+	Xu et al., 2002 Metherell et al., 2005 Fagerberg et al., 2014
Breast	+++	+++	-	Metherell et al., 2005
Ovary	+++	+	+	Fagerberg et al., 2014
Endometrium	+	+	+	Fagerberg et al., 2014 Fagerberg et al., 2014
Digestive tract	+	+	+	Fagerberg et al., 2014
Liver	+	-	-	Metherell et al., 2005
Heart	+	-	-	Metherell et al., 2005 Fagerberg et al., 2014
Skin	+	+	+	Metherell et al., 2005
Brain: Pre-frontal cortex Cerebellum Hippocampus Hypothalamus Spinal cord	-	+ + + + - +	+++ +++ +++ +++ +++ +++	Gardinar et al., 2002 Chan et al., 2009 Fagerberg et al., 2014 Chaly et al., 2016
Pituitary gland	+	-	+++	Chaly et al., 2016
Thyroid gland	+++	-	-	Metherell et al., 2005

210
211
212
213
214
215

216 **MRAPs and the melanocortin system**

217

218 **MRAP and MC₂**

219 MC₂ was initially difficult to express on the surface of cells until an adrenal cell line
220 was utilised, revealing that MRAP is an essential accessory protein (Metherell et al.,
221 2005). The MC₂ receptor only responds to ACTH; ACTH activation of MC₂ results in
222 glucocorticoid-, mineralocorticoid- and steroid-genesis in the adrenal gland. Genetic
223 variants of *MC2R* result in severely impaired glucocorticoid production which is
224 discussed in more detail below. MRAP was found to assist in MC₂ transport from the
225 endoplasmic reticulum to the cell surface (Metherell, et al., 2005), whilst MC₂ has
226 reported to move unassisted to the cell surface (Roy et al., 2007), this finding has been
227 criticised since extremely low endogenous MRAP2 present in HEK293 cells may
228 facilitate the MC₂ movement (Sebag and Hinkle, 2007). MRAP increases both the
229 density of surface MC₂ and the binding affinity of ACTH to MC₂. Measurement of cAMP
230 in response to Gs activation revealed that MRAP- α elicits a 4-fold greater dose
231 response than MRAP- β but the maximal cAMP response was higher in the
232 MC₂/MRAP- β cells compared to the MC₂/MRAP- α (Roy, et al., 2007). The interaction
233 of MRAP and MC₂ also has an effect on receptor internalisation and recycling (Roy,
234 et al., 2011).

235

236 The three zones of the adrenal cortex synthesise different steroids: zona glomerulosa,
237 mineralocorticoids; zona fasciculata, glucocorticoids and zona reticularis, androgens.
238 The binding of ACTH to MC₂ results in the production of cAMP, that in turn stimulates
239 the protein kinase A (PKA) signalling pathway. In the adrenal gland, this signalling
240 pathway is responsible for the transcription of genes necessary for steroid synthesis,
241 such as steroidogenic acute regulatory protein (*StAR*) (Manna, et al., 2003). The
242 interaction of MC₂ with either of the MRAP isoforms and the response variation (Roy,
243 et al., 2007) reflects the physiological requirements of these steroids in different
244 physiological conditions, such as the response to stress or blood pressure changes.

245

246 In addition to *MRAP*, *MRAP2* is expressed in human and rat adrenal glands (Gorrigan,
247 et al., 2011; Fagerberg, et al., 2014). The response of MC₂ depends on which MRAP
248 it is coupled with, *in vitro* studies revealed that MC₂/MRAP2 transiently transfected into
249 HEK293 cells required higher concentrations of ACTH to elicit a response compared
250 to MC₂/MRAP transfected cells (Gorrigan, et al., 2011). *MRAP* and *MRAP2* expression
251 has been described in the foetal adrenal gland suggesting a role of MC₂/MRAP and or
252 MC₂/MRAP2 in the adrenal differentiation and proliferation (Gorrigan, et al., 2011).
253 This is supported by the phenotype of *MRAP*^{-/-} mice which have disrupted adrenal
254 gland development with no apparent zonation in the adrenal cortex, without *in utero*
255 corticosterone supplementation, the majority of these *MRAP*^{-/-} mice died at birth
256 (Novoselova, et al., 2018). *MRAP*^{-/-} mice had disrupted adrenal progenitor cell
257 differentiation due to impaired sonic hedgehog and WNT4/ β -catenin signalling
258 (Novoselova, et al., 2018).

259

260 **MRAPs and other melanocortin receptors**

261

262 The melanocortin receptors are expressed throughout the body and have established
263 roles in different tissues. There is great interest in the involvement of MRAPs and the
264 surface expression of melanocortin receptors, MC_{1,3-5}. These G-coupled protein
265 receptors are activated by melanocortin peptides: proopiomelanocortin (POMC) and
266 the peptides derived from POMC: adrenocorticotrophic hormone (ACTH), alpha-, beta-
267 or gamma-melanocyte stimulating hormone (α -MSH, β -MSH and γ -MSH). ACTH is
268 further cleaved into: ACTH₍₁₋₃₉₎, ACTH₍₁₋₁₇₎, ACTH₍₁₋₁₀₎, α -MSH and deacetyl α -MSH
269 (Wakamatsu, et al., 1997). Unlike MC₂ which only responds to ACTH, these MCs,
270 respond with varying affinity to the endogenous melanocortin peptides and activating
271 different signalling pathways, accounting for MC functional variability. Agouti related
272 protein (AgRP) is an endogenous antagonist of MC₃ and MC₄ (Ollmann, et al., 1997;
273 Fong, et al., 1997). MRAPs regulate both the expression and the response of the other
274 MCs. Although MRAPs had no effect on the MC₁ and MC₃ cell surface expression in
275 *in vitro* studies with CHO cells; MC₁, MC₃, MC₄ and MC₅ became less sensitive to the
276 synthetic α -MSH analogue, NDP-MSH, when co-expressed with either MRAP or
277 MRAP2 (Chan, et al., 2009).

278

279 As described above, MC₂ and the MRAPs are important for adrenal gland
280 development. MC₅ expression has been described in the foetal adrenal gland and is
281 suggested to have a role in foetal development (Nimura, et al., 2006). The role of
282 MRAP and MRAP2 regulating adrenal differentiation, may be through interaction with
283 either MC₂, MC₅ or a heterodimer of both, since heterodimerisation of MC receptors
284 has been reported (Mandrika, et al., 2005).

285

286 MC₁ is well characterised in stimulating melanin synthesis, skin (or retinal)
287 pigmentation occurs through the oxidation of tyrosine resulting in the formation of
288 either eumelanin (brown pigmentation) or pheomelanin (yellowish-red pigmentation).
289 MC₁ is expressed in both melanocytes and keratinocytes, as are POMC, ACTH and
290 the prohormone convertases 1 and 2. α -MSH is the MC₁ agonist, activating cAMP and
291 PKA signalling pathways which ultimately activate the microphthalmia transcription
292 factor (MITF) and tyrosinase transcription (Beaumont, et al., 2009). The resultant
293 pigmentation protects the skin from the DNA damaging effects of the ultraviolet
294 radiation. The variability in skin colour is due to loss of function alleles in *MC1R* single
295 gene mutation which also increase the incidence of skin cancer and melanoma
296 (Sánchez-Laorden, et al., 2007). Whilst mutations of *MRAP2* do not cause
297 disturbances in skin pigmentation, the phenotypic characteristics of *MRAP* mutations
298 have included skin hyperpigmentation, as well as high plasma ACTH concentrations
299 (Jain et al., 2011). Expression of MRAP and MRAP2 in skin is very low (Table 1) a
300 lack of functional MC₂ in *MRAP* mutation may result in higher circulating ACTH and
301 consequently MC₁ hyperactivity impacting upon skin pigmentation.

302
303
304
305
306
307
308
309

The ratio of MRAP2 to the melanocortin receptor may have an important effect on the melanocortin signalling; a 1:4 ratio of MC₄:MRAP2 gave greatest cAMP response to α-MSH whilst a 4:1 ratio of MC₄:MRAP2 reduced cAMP below mock transfected cells (Schonnop, et al., 2016). Further work is required to elucidate the role of MRAP2 on MCs the *MRAP2* construct design, cell type, melanocortin peptide used to stimulate transfected cells and technique to measure MC activation are important factors to consider in interpreting this complex interaction.

310
311
312
313
314
315
316
317
318

In zebrafish two MRAP2 isoforms have been reported (*mrap2a* and *mrap2b*) and the expression of these varies during development; the expression of *mrap2a* was found to be present from day 1 of embryogenesis, as was *mc4r*, however there was little expression of *mrap2b* (Sebag, et al., 2013). Knockdown of *mrap2a* with antisense morpholinos significantly decreased growth, however, when the *mrap2a* was knocked down in *mc4r* null fish the effect was less marked; in the embryo, *mrap2a* inhibits MC₄ signalling. AgRP is an MC₄ antagonist, if both *AgRP* and *mrap2a* were knocked down there was a significant impairment of growth and they are proposed to collaborate inhibiting MC₄ constitutive activity (Sebag, et al., 2013).

319
320
321
322
323

In contrast the *mrap2b* isoform was highly expressed in adult zebrafish, and reduced MC₄ constitutive activity and sensitized MC₄ to the agonist α-MSH; the *mrap2b* has homology to mouse *MRAP2* (Sebag, et al., 2013). It was suggested that this switch in the *mrap2* expression may facilitate a change in metabolism to enable free-feeding once embryonic development was complete.

324 **MRAP and familial glucocorticoid deficiency (FGD)**

325
326
327
328
329
330
331
332
333

Glucocorticoids modulate the stress response through the hypothalamic-pituitary-adrenal (HPA) axis and mediate negative feedback control over the corticotropin releasing hormone (CRH) in the hypothalamus, as well as ACTH release from the pituitary gland. *MRAP2*, expression has been reported in hypothalamus, adrenal and pituitary glands (see table 1) alongside evidence of the melanocortin ligands (Chaly, et al., 2016). These findings suggest the control of the response may be moderated by the melanocortin receptors, with the assistance of the MRAPs, at all three levels of the HPA axis.

334
335
336
337
338
339
340
341
342

Familial glucocorticoid deficiency (FGD) is characterised by a poor response by the adrenal cells to adrenocorticotrophic hormone (ACTH) leading to cortisol deficiency. As described above, the MRAPs have an important role stabilising the MC receptors and facilitating ACTH signalling. FGD is an autosomal recessive disease with 25% of the cases due to different *MC2R* mutations (FGD-1), 5% of cases relate to the steroidogenic acute regulatory protein (*StAR*) but around 50% of the FGD cases had an unidentified genetic background. Single nucleotide polymorphism (SNP) array genotyping of FGD cases revealed that 20% were linked to mutations in the *MRAP*

343 sequence (FGD-2) (Metherell, et al., 2005). The discovery of MRAP as an essential
344 accessory protein for MC₂ prompted further investigations into the mechanisms
345 underlying FGD-1.

346 The *MC2R* coding sequence lies predominantly in one exon, therefore, splicing
347 mutations are very rare and missense mutations preserve a residual function, thus,
348 they are associated with a milder or later onset of the disease. FGD-1 occurs in early
349 childhood with hypoglycaemia, developmental delay and frequent infections, all due
350 to low cortisol levels. The reduced receptor sensitivity to ACTH leads to increased
351 circulating ACTH concentrations which are associated with hyperpigmentation of the
352 skin and tall stature. These latter signs are thought to result from increased MC₁
353 stimulation and estradiol synthesis by excessive circulatory ACTH levels (Imamine, et
354 al., 2005).

355 The cell lines HEK293 and CHO do not express melanocortin receptors and provide
356 a useful *in vitro* model for transfection with the MRAPs and the MCs. Chung and
357 colleagues investigated 24 reported MC₂ polymorphisms with MRAP using cAMP
358 reporter assays and cell surface analyses, they found that two thirds of these SNP
359 mutations impaired MC₂ receptor transport to the cell surface, despite interacting with
360 MRAP, and resulted in a failure of MC₂ receptor to respond to ACTH (Chung, et al.,
361 2008).

362
363 Unlike *MC2R* mutations, almost all the mutations occurring in the *MRAP* are
364 homozygous non-sense or splice-site mutations which cause severe truncation or
365 ablation of the MRAP protein (Jain, et al., 2011), (See table 2). Those mutations were
366 found to be associated with severe glucocorticoid deficiency immediately after birth or
367 in early infancy with seizures due to hypoglycaemia. Cases of the FGD-2 have been
368 reported where there is a later onset and a milder presentation of the symptoms.
369 Sequencing the genome of individuals with later onset glucocorticoid deficiency
370 revealed a homozygous missense (c.175T>G) mutation that caused an aspartame to
371 tyrosine substitution at position 59 (N59Y). Whilst another case was reported a
372 missense (c.76T>C) which led to an alanine to valine substitution at position 26 (V26A)
373 (Hughes, et al., 2010).

374
375 *MRAP*^{-/-} mice do not survive after birth without the intervention of *in utero*
376 corticosterone and had impaired adrenal gland development (Novoselova, et al.,
377 2018). MRAP is likely to have a major role in adrenal gland development and the
378 severity of the FGD-2 could be due to underdevelopment of this gland. Although
379 *MRAP2* is also expressed in the adrenal gland, it did not seem to compensate for
380 absent or mutated *MRAP* and therefore, might not have a role in the pathogenesis of
381 FGD. However, as there are still about 50% of FGD cases with unidentified mutations,
382 further nucleotide sequencing and genome analysis of FGD individuals may reveal a
383 role of MRAP2.

384
385 Whilst *MRAP* genetic mutations leading to glucocorticoid deficiency are rare, the use
386 of synthetic glucocorticoids for inflammatory disorders and autoimmune diseases is

387 widespread. A side-effect of synthetic glucocorticoid treatment is adrenal insufficiency,
 388 characterised by the failure of the adrenal gland to respond to ACTH. In a rat model,
 389 five day exposure of methylprednisolone reduced ACTH and corticosterone
 390 concentrations and downregulated both *MRAP* and *StAR* expression (Spiga, et al.,
 391 2020).

392
 393

394 Table 2: Human *MRAP* mutations which have been described to be associated with
 395 familial glucocorticoid deficiency-type 2. * This mutation in *MRAP* sequence causes
 396 primary adrenal insufficiency.

397

Type of mutation	Codon substitution- Codon number	Amino acid substitution	FGD-2 Mutation phenotype	Reference
Missense	G >A-1	Met1Ile	Disruptive	(Metherell, et al., 2005)
Missense	C >A-11	Tyr11Termination	Disruptive	(Metherell, et al., 2005)
Missense	T >C-26	Val26Ala	Disruptive	(Hughes, et al., 2010)
Missense	T >G-59	Tyr59 Asp	Disruptive	(Hughes, et al., 2010)
Missense	T >C-76	Val76Ala	Disruptive	(Hughes, et al., 2010)
Deletion	c.-17_23del	-	Disruptive	(Modan-Moses, et al., 2006)
Deletion	p.R451W in CYP11A1	-	*Disruptive (Primary adrenal insufficiency)	(Guran, et al., 2016)
Deletion	c.IVS3ds+1delG	-	Disruptive	(Metherell, et al., 2005)
Deletion	c.130del	Val44insTer	Disruptive	(Metherell, et al., 2005)

398
 399

400 **MRAP2 and Appetite control**

401

402 Appetite control comprises a complicated neuroendocrine circuit involving several
403 hormones, the sympathetic and parasympathetic nervous system and some elements
404 of the limbic system. MC₄ regulates energy expenditure and appetite control and has
405 been found in areas of the brain associated with energy homeostasis: *MC4R* mRNA
406 has been detected in the paraventricular nucleus (PVN), ventromedial, medial preoptic
407 and the supraoptic nucleus of the hypothalamus; all of which are integrated in
408 controlling feeding behaviour (Siljee, et al., 2013). In the PVN, *MC4R* was detected in
409 transcription factor single minded 1 (*SIM1*⁺) expressing neurones, a population of
410 neurones known to regulate food intake and mediate MC₄ signalling through a
411 glutamatergic pathway (Xu, et al., 2013). The MC₄ agonist setmelanotide is in clinical
412 trials for treating severe obesity with underlying genetic causes (Kühnen, et al., 2016;
413 Clément, et al., 2018). *MC4R*^{-/-} transgenic mice are severely obese and studies with
414 these animals supported the involvement of MC₄ in appetite control, they had
415 hyperphagia and were non-responsive to melanotan II (MTII), an α -MSH analogue
416 (Marsh, et al., 1999). α -MSH is released by POMC neurons in the arcuate nucleus in
417 response to sensory input from the periphery, this in turn stimulates PVN-MC₄
418 signalling leading to reduced appetite. Whilst leptin hunger signalling to the
419 counteracting agouti-related peptide neurons causes the inactivation of the same
420 PVN, leading to increased appetite. This is a simplified account of how MC₄ is involved
421 in controlling feeding behaviour.

422

423 *MRAP2* was found to be expressed at high levels in the brain and in areas that are
424 associated with appetite control, where MC₄ has been comprehensively described
425 (Liang et al., 2018; Bruschetta et al., 2018; Siljee et al 2013). Similar to transgenic
426 *MC4R*^{-/-} mice, both whole body *MRAP2*^{-/-} and brain targeted *MRAP2*^{-/-} mice were
427 morbidly obese, however, *MRAP2*^{-/-} mice do not show signs of hyperphagia or reduced
428 energy consumption (locomotion and body temperature), they have normal expression
429 of *POMC* but reduced *AgRP* levels; and they respond to MTII treatment (Asai, et al.,
430 2013). Deletion of both *MC4R* and *MRAP2* produced mice that were less obese than
431 single deletion *MC4R*^{-/-} mice. Exposure of *MC4R/MRAP2* transfected cells with α -MSH
432 increases cAMP production compared to *MC4R* expressing cells, further suggesting
433 an interaction between MC₄ and MRAP2 (Asai, et al., 2013). MRAP2 was also found
434 to interact with MC₃, cAMP production increased in *MC3R /MRAP2* transfected cells
435 in response to α -MSH compared to *MC3R* expressing cells (Asai, et al., 2013).

436 MRAP2 might be involved in an energy homeostasis/feeding behaviour circuit
437 independently of MC₄. MC₃ is perceived to have a minor role in energy homeostasis,
438 *MC3R* knockout mice have an obese phenotype but with a reduced lean body mass
439 and reduced appetite (Chen, et al., 2000). *MC3R* is expressed on the POMC neurons
440 in the arcuate nucleus and thought to be exerting autocrine feedback control (Lee, et
441 al., 2008). *MC3R*, *MC4R* and *MRAP2* have been observed in chicken hypothalamus;
442 chicken (c)*MRAP* (a homolog of *MRAP2*) was found to enhance the constitutive

443 activity of ACTH treated c *MC3R* /c *MC4R* transfected cells in a CRE-luciferase
444 reporter assay (Zhang, et al., 2017).

445

446 **MRAP2 interactions with non-melanocortin receptors**

447

448 The role of MRAP2 in mediating energy homeostasis and feeding behaviour extends
449 beyond the melanocortin receptors. *MRAP2* is expressed in the AgRP neurones,
450 aforementioned AgRP is an MC₄ antagonist; MRAP2 may regulate the AgRP neuronal
451 activity, since deletion of *MRAP2* resulted in reduced *AgRP* expression and inhibited
452 AgRP orexigenic activity in response to starvation (Srisai, et al., 2017). The MRAP2-
453 AgRP effect may explain the reduced food intake reported in *MRAP2*^{-/-} mice. GHSR-
454 1a (growth hormone secretagogue receptor 1a) is a receptor found in the arcuate
455 nucleus of the hypothalamus and binds ghrelin, which is released by the stomach,
456 sending sensory input to stimulate feeding via AgRP neurones. Srisai et al described
457 MRAP2 to enhance the signalling of GHSR-1a (Srisai, et al., 2017), via Gα_{q/11}
458 however, MRAP2 independently inhibits β-arrestin recruitment and signalling (Rouault
459 et al., 2020). Whilst MRAP2 was found to stimulate GHSR-1 and thus feeding, it was
460 also found to induce feeding by a counteracting mechanism, suppressing the
461 prokineticin receptor 1 (PKR-1). *PKR-1* has a wide tissue distribution and mediates
462 several functions including suppressing food intake. Measurement of surface density
463 and surface expression of tagged-PKR-1 in *MRAP2* positive and negative cells
464 revealed lower PKR-1 surface expression in MRAP2 expressing cells; MRAP2 may
465 not contribute to PKR-1 transport to the cell surface (Chaly, et al., 2016). In the
466 presence of MRAP2, PKR-1 was found to have reduced signalling, as lower production
467 of cAMP and IP₃ were observed, respectively in the CRE-luciferase and IP-One
468 assays (Chaly, et al., 2016).

469

470 An MRAP2/orexin receptor (OX1R) interaction has been described, OX1R is a
471 hypothalamic receptor expressed in the PVN which stimulates food intake. MRAP2
472 was found to downregulate the surface expression of *OX1R* and inhibit OX1R
473 signalling (Rouault et al., 2017). This study mapped the MRAP2 domains and found
474 the C-terminal domain of MRAP2 inhibited signalling of both OX1R and PKR-1. The
475 MRAP2 N-terminal 23-33 domain was involved in inhibiting the OX1R transport to the
476 cell surface; and the N-terminal 34-43 domain was suggested to exert negative
477 regulatory control over the MRAP2 activity (Rouault, et al., 2017). A distinct region of
478 the MRAP2 N-terminus, from that required for OX1R and PKR-1, is suggested to
479 regulate GHSR-1a signalling (Rouault et al., 2020).

480

481

482 **MRAP2 and obesity**

483

484 Polygenic obesity is affected by genetics, life-style and environmental factors which
485 predispose to increased appetite and reduced energy expenditure; whole genome and
486 exome sequencing have been invaluable to identify genetic factors. Monogenic

487 obesity has a negligible environmental influence, obesity is usually very severe and
488 occurs in early childhood. A role for MRAP2 in the pathogenesis of obesity was
489 suggested following a realisation that it regulates MC₄, which, as discussed above, is
490 essential for energy homeostasis and feeding behaviours. MRAP2 knockout mice
491 have severe early onset obesity (Asai, et al., 2013). Asai et al sequenced the MRAP2
492 coding regions and the intron-exon boundaries, in both adults and children with
493 obesity. They discovered four genetic variants, one which resulted in a non-functional
494 MRAP2 (E24X, see Table 3). The other three rare variants were towards the C-
495 terminus and considered to have less impact on obesity (Asai et al., 2013 - see table
496 3). The different functional domains in the MRAP2 observed by Rouault et al., (2017),
497 might suggest that these variants could influence interactions of MRAP2 / MC₄. Eight
498 more genetic variants of MRAP2 have been detected (3 intronic; 2 synonymous; 2
499 coding and one synonymous; see table 3) following mutational sequencing of the
500 MRAP2 coding region of extremely obese individuals (Schonnop, et al., 2016), which
501 were absent in the control group. This study also reported reduced MC₄ signalling
502 when co-transfected with the nonsynonymous MRAP2 mutant (p.Gln174Arg) in
503 HEK293 cells (Schonnop, et al., 2016). Studies of the 6q14.1-6q16.3 region revealed
504 SIM1⁺ and MRAP2 variants in patients with Prader-Willi like-syndrome (Geets, et al.,
505 2016).

506

507 The mechanism by which MRAP2 deletion causes obesity is not clearly understood
508 and predicted to be due to effects on MC₄. However, RT-qPCR analysis of
509 hypothalamus from MRAP2^{tm1a/tm1a} (transgenic mice with targeted exon 4 deletion)
510 showed normal MC4R PVN content. In contrast, there was a 50 % reduction in the
511 SIM1⁺ in the PVN and decreased PVN expression of oxytocin, arginine vasopressin,
512 corticotrophin-releasing hormone and thyrotropin-releasing hormone (Novoselova, et
513 al., 2016). MRAP2^{tm1a/tm1a} mice also had raised cholesterol and lipoprotein (HDL and
514 LDL) concentrations, consistent with observations in MC₄ deficient mice; there is a
515 suggestion that SIM1⁺ is either activated downstream of MC₄ (Novoselova, et al.,
516 2016) or mediates the MC₄ function within the hypothalamic PVN (Xu, et al., 2013).

517

518 Although mutations in the MRAP2 cause an early onset obesity like MC4R mutations,
519 Asai et al. (2013) observed no change in the feeding or energy expenditure patterns
520 in the MRAP2 knockouts compared to the MC4R knockouts. The transgenic mice used
521 by Novoselova et al which have a different genetic background, show some changes
522 in the feeding behaviour. These two reports preceded reports that MRAP2 interacts
523 with the non-melanocortin GPCRs: GHSR-1a, ORX1 and PKR-1. This raises a
524 possibility that, the obesity pathologies observed in MRAP2 transgenic mice could
525 involve pathways outside of melanocortin signalling.

526

527 The MRAP2 exon regions were sequenced in a cohort of 9418 subjects, and 23 rare
528 heterozygous variants were identified which independently increase the risk of obesity
529 (Baron, et al., 2019). To elucidate a molecular mechanism these variants were co-
530 transfected in CHO cells with MC4R and the cAMP production recorded in response

531 to ACTH or α -MSH. Seven *MRAP2* variants (c.3-7 del, G31V, F62V, F62C, N77S,
532 K102* and P195L) decreased cAMP produced in response to ACTH or α -MSH. The
533 variants C5-5del, c.3-7 del, G31V, F62V, F62C, N77S, K102* and P195L were
534 associated with obesity (table 3), the authors remarked that 75% of the carriers of
535 *MRAP* variants also displayed an abnormal eating behaviour (Baron, et al., 2019).
536 *Lep*, *LepR*, *MC4R*, *PCSK1*, *POMC* and *SIM1* are implicated in monogenic obesity,
537 however, individuals with *MRAP2* variants also have higher hypertension and
538 hyperglycaemia (Baron, et al., 2019). As mentioned above, Srisai *et al* reported that
539 *MRAP2* enhances the ghrelin signalling through GHSR-1a, ghrelin receptor has been
540 described to affect blood pressure regulation (Mao, et al., 2016) and further
541 investigation of *MRAP2* and ghrelin signalling is required to understand the
542 hypertension phenotype described in these individuals.

543

544 Table 3: *MRAP2* mutations associated with obesity in humans. 1- *MRAP2*
545 heterozygous mutations detected using coding/intron-exon sequencing of *MRAP2*
546 gene in coding exons; 2- two cohort studies with severe obesity; 3- patients with
547 Prader-Willi like phenotype; 4- Genome -wide association study for severe early -onset
548 obesity.

549

550

Type of mutation	Codon substitution-Codon number	Amino acid substitution	Mutation phenotype	Reference
Missense	G >A-3	Ala3Thr	Uncertain significance	(Baron, et al., 2019)
Missense	G >T-3	Ala3Ser	Uncertain significance	(Baron, et al., 2019)
Missense	C >G-13	Gln13Glu	Uncertain significance	(Baron, et al., 2019)
Nonsense	G >T-24	Glu24Term	Disruptive	(Asai, et al., 2013)
Missense	G >T-31	Gly31Val	Disruptive	(Baron, et al., 2019)
Missense	C >T-32	Pro32Leu	Uncertain significance	(Baron, et al., 2019)
Missense	G >T-40	Ala40Ser	Non-pathogenic	(Geets, et al., 2016)
Missense	T >G-62	Phe62Cys	Disruptive	(Baron, et al., 2019)
Missense	A >G-77	Asn77Ser	Disruptive	(Baron, et al., 2019)
Missense	A >T-88	Asn88Tyr	Non-pathogenic	(Asai, et al., 2013)
Missense	T >C-91	Val91Ala	Uncertain significance	(Baron, et al., 2019)
Missense	G >C-99	Glu99Gln	Uncertain significance	(Baron, et al., 2019)
Missense	A >T-102	Lys102X	Disruptive	(Baron, et al., 2019)
Missense	A >G-113	Arg113Gly	Uncertain significance	(Baron, et al., 2019)
Missense	T >G-114	Ser114Ala	Uncertain significance	(Baron, et al., 2019)
Missense	C >G-115	Leu115Val	Non-pathogenic	(Asai, et al., 2013)
Missense	A >G-121	Asn121Ser	Uncertain significance	(Baron, et al., 2019)
Missense	C >T-125	Arg125Cys	Uncertain significance	(Baron, et al., 2019)
Missense	G >A-125	Arg125His	Non-pathogenic	(Schonnop, et al., 2016)
Missense	C >T-133	His133Tyr	Uncertain significance	(Baron, et al., 2019)
Missense	G >A-137	Ala137Thr	Non-Pathogenic	(Schonnop, et al., 2016)

Missense	T > C-162	Met162Thr	Uncertain significance	(Baron, et al., 2019)
Missense	A > G-174	Gln174Arg	Non-pathogenic	(Schonnop, et al., 2016)
Missense	A > G-193	Thr193Ala	Uncertain significance	(Baron, et al., 2019)
Missense	C > T-195	Pro195Leu	Disruptive	(Baron, et al., 2019)
Missense	G > T-203	Asp203Tyr	Uncertain significance	(Baron, et al., 2019)
Deletion	c.-5_5del	-	Loss of function	(Baron, et al., 2019)
Deletion	c.-3_7del	-	Loss of function	(Baron, et al., 2019)

551
552
553
554

MRAP and insulin signalling

555 The first description of MRAP came from murine 3T3L1 adipocytes; a novel low
556 molecular weight protein was found in differentiated adipocytes but not pre-adipocytes,
557 and in both in brown and white adipose tissue. Initially named fat tissue low molecular
558 weight protein (FALP) (Xu, et al., 2002) it was subsequently renamed MRAP after
559 recognition of the MC₂ interaction (Metherell, et al., 2005). PPAR γ , a regulator of
560 adipocyte differentiation, activates transcription of *MRAP* (Kim, et al., 2013). MRAP
561 was observed in adipocytes at the peri-nuclear membrane, but if adipocytes were
562 exposed to insulin the distribution changed, with discrete MRAP specks distributed
563 throughout the cytoplasm (Xu, et al., 2002).

564 Baron et al reported that siRNA *MRAP2* knockdown in EndoC- β H1 cells reduced
565 insulin secretion (Baron, et al., 2019). In adipocytes, MRAP is required for lipolytic
566 responses to ACTH through MRAP interaction with G α s (Zhang, et al., 2018).
567 Increased insulin sensitivity in response to a high fat diet has been reported in
568 transgenic mice with *MRAP* overexpression in adipose tissue, correlating with
569 decreased plasma triglyceride and cholesterol concentrations. Insulin resistance and
570 glucose intolerance has also been described in *MRAP2* knockout mice (Rouault, et
571 al., 2017). Further research is required to elucidate these pathways.

572
573
574

Inflammation

575 MC₁ and MC₃ signalling through α -MSH and ACTH mediate anti-inflammatory
576 responses downregulating and inhibiting the release of proinflammatory cytokines:
577 TNF- α , INF- γ , IL-6; inducible nitric oxide synthase and the chemokine receptors CXCL
578 and 2 whilst stimulating IL-1 release (Catania, et al., 1998; Catania, et al., 2004;
579 Getting, et al., 2003). MC₃ therapeutics targeted for rheumatoid arthritis and other
580 chronic inflammatory conditions report side effects of hyperpigmentation and
581 increased melanoma risk, through hyperstimulation of MC₁. Adrenal inflammation is
582 associated with side effects of synthetic corticosteroids where MRAP2 is
583 downregulated (Spiga, et al., 2020). Additional investigations may determine whether
584 either MRAP isoform has an anti-inflammatory role.

585
586
587
588
589

MRAP2- Single nucleotide variations related to cancer

590 The BioMuta single-nucleotide variation (SNV) and disease database revealed
591 *MRAP2* SNV to be linked to different types of cancers with varying frequencies. High
592 frequency of SNV was seen with melanomas and uterine cancers followed by a lower
593 variation frequency seen in colorectal, lung, stomach and liver cancers (Simonyan,
594 and Mazumder, 2014). As discussed above *MC1R* mutations are associated with
595 melanoma (Sánchez-Laorden, et al., 2007), although not affecting MC_1 surface
596 expression, *MRAP2* reduces responsiveness of MC_1 to the synthetic α -MSH agonist
597 NDP-MSH and affects receptor signalling (Chan, et al., 2009). Other types of cancers
598 that were showed a lower *MRAP2* SNV include breast, thyroid, prostate, pancreas,
599 urinary tract and brain cancers (Simonyan, and Mazumder, 2014). SNV that are linked
600 to *MRAP2* over-expression included liver, lung and pancreatic cancers, however, SNV
601 leading to *MRAP2* suppression included breast, thyroid and prostate cancers. The
602 tissue distribution of *MRAP2* is shown in table 1; *MRAP2* has not been reported in
603 healthy human liver, thyroid or urinary tract. The association of *MRAP2* to a wide
604 variety of cancers is of interest. However, the potential pathogenesis and involvement
605 of the melanocortin receptors or other GPCRs needs to be elucidated.

606
607

Female fertility

609 Recently in this journal, *MRAP2* was described to be significantly up-regulated in
610 women with unexplained infertility (D'Aurora, et al., 2019). *MRAP- α* , *MRAP- β* and
611 *MRAP2* have low expression in the endometrium (Table 1). Hypertrophy of the uterine
612 gland epithelium was observed in women with unexplained infertility. The increased
613 *MRAP2* expression suggests that *MRAP2* might influence the stability of the
614 endometrium (D'Aurora, et al., 2019); understanding the roles of *MRAP* and *MRAP2*
615 in the uterus and female reproductive axis may reveal a role for melanocortin signalling
616 in infertility.

617
618
619

Summary

620
621
622
623
624
625
626
627
628

In summary, genetic variants of *MRAP* are associated with severe familial
glucocorticoid deficiency, however evidence is emerging that synthetic glucocorticoids
may also disrupt ACTH and *MRAP* expression resulting in adrenal inflammation.
MRAP2 genetic variants are associated with severe obesity but the phenotypes are
subtly different to the phenotypes observed with *MC4R* genetic variants. There is a
great deal of interest in the development of melanocortin therapeutics to treat obesity,
as evidenced by setmelanotide, and fully understanding the role of *MRAP2* and the

629 interactions with both melanocortin receptors and other GCPR would be of great value.
630 The use of the *MRAP2* knockout and transgenic models have aided understanding of
631 interactions with the different MCs in metabolic regulation, feeding behaviour and
632 energy homeostasis. *MRAP* is detected in areas with absent or extremely low MC₂
633 expression, potentially it also may bind to non-melanocortin receptors. Further studies
634 with transgenic models and insights from studies of genetic variants may provide
635 further insights into the MRAP physiological processes.

636

637

638 **Acknowledgement**

639

640 This review and the corresponding Gene Wiki article are written as part of the
641 GeneWiki Review series--a series resulting from a collaboration between the journal
642 GENE and the Gene Wiki Initiative. The Gene Wiki Initiative is supported by National
643 Institutes of Health (GM089820). Additional support for Gene Wiki Reviews is provided
644 by Elsevier, the publisher of GENE. The corresponding Gene Wiki entries for this
645 review can be found here:

646 MRAP: https://en.wikipedia.org/wiki/Melanocortin_2_receptor_accessory_protein

647 MRAP2: <https://en.wikipedia.org/wiki/MRAP2>.

648

649

650

651 **References**

- 652 Adan, R.A.H., Tiesjema, B., Hillebrand, J.J.G., La Fleur, S.E., Kas, M.J.H. and De
653 Krom, M., (2006). The MC4 receptor and control of appetite. *British Journal of*
654 *Pharmacology*, **149**(7), 815–827.
- 655 Agulleiro, M.J., Roy, S., Sánchez, E., Puchol, S., Gallo-Payet, N. and Cerdá-Reverter,
656 J.M., (2010). Role of melanocortin receptor accessory proteins in the function of
657 zebrafish melanocortin receptor type 2. *Molecular and Cellular Endocrinology*,
658 **320**(1–2), 145–152.
- 659 Asai, M., Ramachandrappa, S., Joachim, M., Shen, Y., Zhang, R., Nuthalapati, N.,
660 Ramanathan, V., Strohlic, D.E., Ferket, P., Linhart, K., Ho, C., Novoselova, T.
661 V., Garg, S., Ridderstråle, M., Marcus, C., Hirschhorn, J.N., Keogh, J.M.,
662 O’Rahilly, S., Chan, L.F., et al., (2013). Loss of function of the melanocortin 2
663 receptor accessory protein 2 is associated with mammalian obesity. *Science*,
664 **341**(6143), 275–278.
- 665 Baron, M., Maillet, J., Huyvaert, M., Dechaume, A., Boutry, R., Loïselle, H., Durand,
666 E., Toussaint, B., Vaillant, E., Philippe, J., Thomas, J., Ghulam, A., Franc, S.,
667 Charpentier, G., Borys, J.M., Lévy-Marchal, C., Tauber, M., Scharfmann, R.,
668 Weill, J., et al., (2019). Loss-of-function mutations in MRAP2 are pathogenic in
669 hyperphagic obesity with hyperglycemia and hypertension. *Nature Medicine*,
670 **25**(11), 1733–1738.
- 671 Beaumont, K.A., Liu, Y.Y. and Sturm, R.A., (2009). The Melanocortin-1 Receptor
672 Gene Polymorphism and Association with Human Skin Cancer. *Progress in*
673 *Molecular Biology and Translational Science*, **88**, 85–153.
- 674 Bruschetta, G., Kim, J.D., Diano, S. and Chan, L.F., (2018). Overexpression of
675 melanocortin 2 receptor accessory protein 2 (MRAP2) in adult paraventricular
676 MC4R neurons regulates energy intake and expenditure. *Molecular Metabolism*,
677 **18**, 79–87.
- 678 Catania, A., Garofalo, L., Cutuli, M., Gringeri, A. and Santagostino, E. et al., (1998).
679 Melanocortin peptides inhibit production of proinflammatory cytokines in blood of
680 HIV-infected patients. *Peptides*, **19**(6), 1099–1104.
- 681 Catania, A., Gatti, S., Colombo, G. and Lipton, J.M., (2004). Targeting melanocortin
682 receptors as a novel strategy to control inflammation. *Pharmacological reviews*,
683 **56**(1), 1–29.
- 684 Chaly, A.L., Srisai, D., Gardner, E.E. and Sebgag, J.A., (2016). The melanocortin
685 receptor accessory protein 2 promotes food intake through inhibition of the
686 prokineticin receptor-1. *eLife*, **5**, e12397.
- 687 Chan, L.F., Webb, T.R., Chung, T.T., Meimaridou, E. and Cooray, S.N. et al., (2009).
688 MRAP and MRAP2 are bidirectional regulators of the melanocortin receptor
689 family. *Proceedings of the National Academy of Sciences*, **106**(15), 6146–6151.
- 690 Chen, A.S., Marsh, D.J., Trumbauer, M.E., Frazier, E.G., Guan, X.M., Yu, H.,
691 Rosenblum, C.I., Vongs, A., Feng, Y., Cao, L., Metzger, J.M., Strack, A.M.,
692 Camacho, R.E., Mellin, T.N., Nunes, C.N., Min, W., Fisher, J., Gopal-Truter, S.,
693 MacIntyre, D.E., et al., (2000). Inactivation of the mouse melanocortin-3 receptor
694 results in increased fat mass and reduced lean body mass. *Nature Genetics*,
695 **26**(1), 97–102.
- 696 Chung, T.T., Webb, T.R., Chan, L.F., Cooray, S.N., Metherell, L.A., King, P.J.,
697 Chapple, J.P. and Clark, A.J.L., (2008). The majority of adrenocorticotropin
698 receptor (melanocortin 2 receptor) mutations found in familial glucocorticoid
699 deficiency type 1 lead to defective trafficking of the receptor to the cell surface.
700 *Journal of Clinical Endocrinology and Metabolism*, **93**(12), 4948–4954.

701 Clark, A.J.L. and Chan, L., (2019). Stability and Turnover of the ACTH Receptor
702 Complex. *Frontiers in Endocrinology*, **10**:491.

703 Clément, K., Biebermann, H., Farooqi, I.S., Van Der Ploeg, L., Wolters, B., Poitou, C.,
704 Puder, L., Fiedorek, F., Gottesdiener, K., Kleinau, G., Heyder, N., Scheerer, P.,
705 Blume-Peytavi, U., Jahnke, I., Sharma, S., Mokrosinski, J., Wiegand, S., Müller,
706 A., Weiß, K., et al., (2018). MC4R agonism promotes durable weight loss in
707 patients with leptin receptor deficiency. *Nature Medicine*, **24**(5), 551–555.

708 D'Aurora, M., Romani, F., Franchi, S., Diomede, F., Merciaro, I., Impicciatore, G.G.,
709 Trubiani, O., Stuppia, L., Tiboni, G.M. and Gatta, V., (2019). MRAP2 regulates
710 endometrial receptivity and function. *Gene*, **703**, 7–12.

711 Dores, R.M., Liang, L., Davis, P., Thomas, A.L. and Petko, B., (2016). Melanocortin
712 receptors: Evolution of ligand selectivity for melanocortin peptides. *Journal of*
713 *Molecular Endocrinology*, **56**(4), T119–T133.

714 Fagerberg, L., Hallstrom, B.M., Oksvold, P., Kampf, C., Djureinovic, D., Odeberg, J.,
715 Habuka, M., Tahmasebpour, S., Danielsson, A., Edlund, K., Asplund, A., Sjostedt,
716 E., Lundberg, E., Szigyarto, C.A.K., Skogs, M., Ottosson Takanen, J., Berling, H.,
717 Tegel, H., Mulder, J., et al., (2014). Analysis of the human tissue-specific
718 expression by genome-wide integration of transcriptomics and antibody-based
719 proteomics. *Molecular and Cellular Proteomics*, **13**(2), 397–406.

720 Fong, T.M., Mao, C., MacNeil, T., Kalyani, R., Smith, T., Weinberg, D., Tota, M.R. and
721 Van Der Ploeg, L.H.T., (1997). ART (protein product of agouti-related transcript)
722 as an antagonist of MC-3 and MC-4 receptors. *Biochemical and Biophysical*
723 *Research Communications*, **237**(3), 629–631.

724 Geets, E., Zegers, D., Beckers, S., Verrijken, A., Massa, G., Van Hoorenbeeck, K.,
725 Verhulst, S., Van Gaal, L. and Van Hul, W., (2016). Copy number variation (CNV)
726 analysis and mutation analysis of the 6q14.1-6q16.3 genes SIM1 and MRAP2 in
727 Prader Willi like patients. *Molecular Genetics and Metabolism*, **117**(3), 383–388.

728 Getting, S.J., Christian, H.C., Lam, C.W., Gavins, F.N.E., Flower, R.J., Schiöth, H.B.
729 and Perretti, M., (2003). Redundancy of a functional melanocortin 1 receptor in
730 the anti-inflammatory actions of melanocortin peptides: studies in the recessive
731 yellow (e/e) mouse suggest an important role for melanocortin 3 receptor. *Journal*
732 *of immunology*, **170**(6), 3323–3330.

733 Gibbison, B., Spiga, F., Walker, J.J., Russell, G.M., Stevenson, K., Kershaw, Y., Zhao,
734 Z., Henley, D., Angelini, G.D. and Lightman, S.L., (2015). Dynamic pituitary-
735 adrenal interactions in response to cardiac surgery. In *Critical Care Medicine*.
736 **43**(4), 791–800.

737 Gorrigan, R.J., Guasti, L., King, P., Clark, A.J. and Chan, L.F., (2011). Localisation of
738 the melanocortin-2-receptor and its accessory proteins in the developing and
739 adult adrenal gland. *Journal of Molecular Endocrinology*, **46**(3), 227–232.

740 Guran, T., Buonocore, F., Saka, N., Ozbek, M.N., Aycan, Z., Bereket, A., Bas, F.,
741 Darcan, S., Bideci, A., Guven, A., Demir, K., Akinci, A., Buyukinan, M., Aydin,
742 B.K., Turan, S., Agladioglu, S.Y., Atay, Z., Abali, Z.Y., Tarim, O., et al., (2016).
743 Rare causes of primary adrenal insufficiency: Genetic and clinical
744 characterization of a large nationwide cohort. *Journal of Clinical Endocrinology*
745 *and Metabolism*, **101**(1), 284–292.

746 Hughes, C.R., Chung, T.T., Habeb, A.M., Kelestimur, F., Clark, A.J.L.L. and Metherell,
747 L.A., (2010). Missense mutations in the melanocortin 2 receptor accessory protein
748 that lead to late onset familial glucocorticoid deficiency type 2. *Journal of Clinical*
749 *Endocrinology and Metabolism*, **95**(7), 3497–3501.

750 Imamine, H., Mizuno, H., Sugiyama, Y., Ohro, Y., Sugiura, T. and Togari, H., (2005).

751 Possible relationship between elevated plasma ACTH and tall stature in familial
752 glucocorticoid deficiency. *Tohoku Journal of Experimental Medicine*, **205**(2), 123–
753 131.

754 Jain, V., Metherell, L.A., David, A., Sharma, R., Sharma, P.K., Clark, A.J.L. and Chan,
755 L.F., (2011). Neonatal presentation of familial glucocorticoid deficiency resulting
756 from a novel splice mutation in the melanocortin 2 receptor accessory protein.
757 *European Journal of Endocrinology*, **165**(6), 987–991.

758 Kay, E.I., Botha, R., Montgomery, J.M. and Mountjoy, K.G., (2015). HMRAP α , but not
759 hMRAP2, enhances hMC4R constitutive activity in HEK293 cells and this is not
760 dependent on hMRAP α induced changes in hMC4R complex N-linked
761 glycosylation. *PLoS ONE*, **10**(10), e0140320.

762 Kim, N.S., Kim, Y.J., Cho, S.Y., Lee, T.R. and Kim, S.H., (2013). Transcriptional
763 activation of melanocortin 2 receptor accessory protein by PPAR γ in adipocytes.
764 *Biochemical and Biophysical Research Communications*, **439**(3), 401–406.

765 Kühnen, P., Clément, K., Wiegand, S., Blankenstein, O., Gottesdiener, K., Martini,
766 L.L., Mai, K., Blume-Peytavi, U., Grüters, A. and Krude, H., (2016).
767 Proopiomelanocortin Deficiency Treated with a Melanocortin-4 Receptor Agonist.
768 *New England Journal of Medicine*, **375**(3), 240–246.

769 Lee, M., Kim, A., Conwell, I.M., Hruby, V., Mayorov, A., Cai, M. and Wardlaw, S.L.,
770 (2008). Effects of selective modulation of the central melanocortin-3-receptor on
771 food intake and hypothalamic POMC expression. *Peptides*, **29**(3), 440–447.

772 Liang, J., Li, L., Jin, X., Xu, B. and Pi, L. et al., (2018). Pharmacological effect of human
773 melanocortin-2 receptor accessory protein 2 variants on hypothalamic
774 melanocortin receptors. *Endocrine*, **61**(1), 94–104.

775 Liu, Y., Smith, L.I., Huang, V., Poon, V., Coello, A., Olah, M., Spiga, F., Lightman, S.L.
776 and Aguilera, G., (2013). Transcriptional regulation of episodic glucocorticoid
777 secretion. *Molecular and Cellular Endocrinology*, **371**(1–2), 62–70.

778 Maben, Z.J., Malik, S., Jiang, L.H. and Hinkle, P.M., (2016). Dual topology of the
779 melanocortin-2 receptor accessory protein is stable. *Frontiers in Endocrinology*,
780 **7**:96.

781 Mandrika, I., Petrovska, R. and Wikberg, J., (2005). Melanocortin receptors form
782 constitutive homo- and heterodimers. *Biochemical and Biophysical Research
783 Communications*, **326**(2), 349–354.

784 Manna, P.R., Eubank, D.W., Lalli, E., Sassone-Corsi, P. and Stocco, D.M., (2003).
785 Transcriptional regulation of the mouse steroidogenic acute regulatory protein
786 gene by the cAMP response-element binding protein and steroidogenic factor 1.
787 *Journal of Molecular Endocrinology*, **30**(3), 381–397.

788 Mao, Y., Tokudome, T. and Kishimoto, I., (2016). Ghrelin and Blood Pressure
789 Regulation. *Current Hypertension Reports*, **18**(2), 1–6.

790 Marsh, D.J., Hollopeter, G., Huszar, D., Laufer, R., Yagaloff, K.A., Fisher, S.L., Burn,
791 P. and Palmiter, R.D., (1999). Response of melanocortin-4 receptor-deficient
792 mice to anorectic and orexigenic peptides. *Nature Genetics*, **21**(1), 119–122.

793 Metherell, L.A., Chapple, J.P., Cooray, S., David, A. and Becker, C. et al., (2005).
794 Mutations in MRAP, encoding a new interacting partner of the ACTH receptor,
795 cause familial glucocorticoid deficiency type 2. *Nature Genetics*, **37**(2), 166–170.

796 Modan-Moses, D., Ben-Zeev, B., Hoffmann, C., Falik-Zaccari, T.C., Bental, Y.A.,
797 Pinhas-Hamiel, O. and Anikster, Y., (2006). Clinical case seminar: Unusual
798 presentation of familial glucocorticoid deficiency with a novel MRAP mutation. In
799 *Journal of Clinical Endocrinology and Metabolism*. **91**(10), 3713–3717.

800 Nimura, M., Udagawa, J., Hatta, T., Hashimoto, R. and Otani, H., (2006). Spatial and

801 temporal patterns of expression of melanocortin type 2 and 5 receptors in the fetal
802 mouse tissues and organs. *Anatomy and Embryology*, **211**(2), 109–117.

803 Novoselova, T. V., Larder, R., Rimmington, D., Lelliott, C. and Wynn, E.H. et al.,
804 (2016). Loss of Mrap2 is associated with Sim1 deficiency and increased
805 circulating cholesterol. *Journal of Endocrinology*, **230**(1), 13–26.

806 Novoselova, T. V., Hussain, M., King, P.J., Guasti, L., Metherell, L.A., Charalambous,
807 M., Clark, A.J.L. and Chan, L.F., (2018). MRAP deficiency impairs adrenal
808 progenitor cell differentiation and gland zonation. *FASEB Journal*, **32**(11), 6186–
809 6196.

810 Ohno, S., Wolf, U. and Atkin, N.B., (1968). Evolution From Fish To Mammals By Gene
811 Duplication. *Hereditas*, **59**(1), 169–187.

812 Ollmann, M.M., Wilson, B.D., Yang, Y.K., Kerns, J.A., Chen, Y., Gantz, I. and Barsh,
813 G.S., (1997). Antagonism of Central Melanocortin receptors in vitro and in vivo by
814 agouti-related protein. *Science*, **278**(5335), 135–138.

815 Rouault, A.A.J., Lee, A.A. and Sebag, J.A., (2017). Regions of MRAP2 required for
816 the inhibition of orexin and prokineticin receptor signaling. *Biochimica et*
817 *Biophysica Acta - Molecular Cell Research*, **1864**(12), 2322–2329.

818 Roy, S., Roy, S.J., Pinard, S., Taillefer, L.D., Rached, M., Parent, J.L. and Gallo-Payet,
819 N., (2011). Mechanisms of melanocortin-2 receptor (MC2R) internalization and
820 recycling in human embryonic kidney (HEK) cells: Identification of key Ser/Thr
821 (S/T) amino acids. *Molecular Endocrinology*, **25**(11), 1961–1977.

822 Roy, S., Roy, S.J., Pinard, S., Agulleiro, M.J., Cerdá-Reverter, J.M., Parent, J.L. and
823 Gallo-Payet, N., (2012). The C-terminal domains of melanocortin-2 receptor
824 (MC2R) accessory proteins (MRAP1) influence their localization and ACTH-
825 induced cAMP production. *General and Comparative Endocrinology*, **176**(2),
826 265–274.

827 Roy, S., Rached, M. and Gallo-Payet, N., (2007). Differential regulation of the human
828 adrenocorticotropin receptor [melanocortin-2 receptor (MC2R)] by human MC2R
829 accessory protein isoforms α and β in isogenic human embryonic kidney 293
830 cells. *Molecular Endocrinology*, **21**(7), 1656–1669.

831 Sánchez-Laorden, B.L., Jiménez-Cervantes, C. and García-Borrón, J.C., (2007).
832 Regulation of human melanocortin 1 receptor signaling and trafficking by Thr-308
833 and Ser-316 and its alteration in variant alleles associated with red hair and skin
834 cancer. *Journal of Biological Chemistry*, **282**(5), 3241–3251.

835 Schonnop, L., Kleinau, G., Herrfurth, N., Volckmar, A.L., Cetindag, C., Müller, A.,
836 Peters, T., Herpertz, S., Antel, J., Hebebrand, J., Biebermann, H. and Hinney, A.,
837 (2016). Decreased melanocortin-4 receptor function conferred by an infrequent
838 variant at the human melanocortin receptor accessory protein 2 gene. *Obesity*,
839 **24**(9), 1976–1982.

840 Sebag, J.A., Zhang, C., Hinkle, P.M., Bradshaw, A.M. and Cone, R.D., (2013).
841 Developmental control of the melanocortin-4 receptor by MRAP2 proteins in
842 zebrafish. *Science*, **341**(6143), 278–281.

843 Sebag, J.A. and Hinkle, P.M., (2007). Melanocortin-2 receptor accessory protein
844 MRAP forms antiparallel homodimers. *Proceedings of the National Academy of*
845 *Sciences of the United States of America*. **104**(51), 20244–20249.

846 Sebag, J.A. and Hinkle, P.M., (2009). Opposite effects of the melanocortin-2 (MC2)
847 receptor accessory protein MRAP on MC2 and MC5 receptor dimerization and
848 trafficking. *Journal of Biological Chemistry*, **284**(34), 22641–22648.

849 Sebag, J.A. and Hinkle, P.M., (2010). Regulation of G protein-coupled receptor
850 signaling: Specific dominant-negative effects of melanocortin 2 receptor

851 accessory protein2. *Science Signaling*, **3**(116), ra28.

852 Siljee, J.E., AUnmehopa, U., Kalsbeek, A., Swaab, D.F., Fliers, E. and Alkemade, A.,
853 (2013). Melanocortin 4 receptor distribution in the human hypothalamus.
854 *European Journal of Endocrinology*, **168**(3), 361–369.

855 Simonyan, V. and Mazumder, R., (2014). High-performance integrated virtual
856 environment (hive) tools and applications for big data analysis. *Genes*, **5**(4), 957–
857 981.

858 Souvorov, a, Kapustin, Y., Kiryutin, B., Chetvernin, V., Tatusova, T. and Lipman, D.,
859 (2010). Gnomon–NCBI eukaryotic gene prediction tool. *National Center for*
860 *Biotechnology Information*, 1-24.

861 Spiga, F., Zhao, Z. and Lightman, S.L., (2020). Prolonged treatment with the synthetic
862 glucocorticoid methylprednisolone affects adrenal steroidogenic function and
863 response to inflammatory stress in the rat. *Brain, Behavior, and Immunity*.
864 **87**:703-714.

865 Srisai, D., Yin, T.C., Lee, A.A., Rouault, A.A.J. and Pearson, N.A. et al., (2017).
866 MRAP2 regulates ghrelin receptor signaling and hunger sensing. *Nature*
867 *Communications*, **8**(1), 713.

868 Valsalan, R., Krishnan, A., Almén, M.S., Fredriksson, R. and Schiöth, H.B., (2012).
869 Early vertebrate origin of melanocortin 2 receptor accessory proteins (MRAPs).
870 *General and Comparative Endocrinology*, **188**(1), 123–132.

871 Västermark, Å. and Schiöth, H.B., (2011). The early origin of melanocortin receptors,
872 agouti-related peptide, agouti signalling peptide, and melanocortin receptor-
873 accessory proteins, with emphasis on pufferfishes, elephant shark, lampreys, and
874 amphioxus. *European Journal of Pharmacology*, **660**(1), 61–69.

875 Venkatesh, B., Lee, A.P., Ravi, V., Maurya, A.K., Lian, M.M., Swann, J.B., Ohta, Y.,
876 Flajnik, M.F., Sutoh, Y., Kasahara, M., Hoon, S., Gangu, V., Roy, S.W., Irimia, M.,
877 Korzh, V., Kondrychyn, I., Lim, Z.W., Tay, B.H., Tohari, S., et al., (2014). Elephant
878 shark genome provides unique insights into gnathostome evolution. *Nature*,
879 **505**(7482), 174–179.

880 Wakamatsu, K., Graham, A., Cook, D. and Thody, A.J., (1997). Characterisation of
881 ACTH Peptides in Human Skin and Their Activation of the Melanocortin-1
882 Receptor. *Pigment Cell Research*, **10**(5), 288–297.

883 Webb, T.R., Chan, L., Cooray, S.N., Cheetham, M.E., Chapple, J.P. and Clark, A.J.L.,
884 (2009). Distinct melanocortin 2 receptor accessory protein domains are required
885 for melanocortin 2 receptor interaction and promotion of receptor trafficking.
886 *Endocrinology*, **150**(2), 720–726.

887 Xu, A., Choi, K.L., Wang, Y., Permana, P.A., Yi Xu, L., Bogardus, C. and Cooper,
888 G.J.S., (2002). Identification of novel putative membrane proteins selectively
889 expressed during adipose conversion of 3T3-L1 cells. *Biochemical and*
890 *Biophysical Research Communications*, **293**(4), 1161–1167.

891 Xu, Yuanzhong, Wu, Z., Sun, H., Zhu, Y., Kim, E.R., Lowell, B.B., Arenkiel, B.R., Xu,
892 Yong and Tong, Q., (2013). Glutamate mediates the function of melanocortin
893 receptor 4 on Sim1 neurons in body weight regulation. *Cell Metabolism*, **18**(6),
894 860–870.

895 Zhang, J., Li, X., Zhou, Y., Cui, L. and Li, J. et al., (2017). The interaction of MC3R
896 and MC4R with MRAP2, ACTH, α -MSH and AgRP in chickens. *Journal of*
897 *Endocrinology*, **234**(2), 155–174.

898 Zhang, X., Saarinen, A.M., Campbell, L.E., De Filippis, E.A. and Liu, J., (2018).
899 Regulation of lipolytic response and energy balance by melanocortin 2 receptor
900 accessory protein (MRAP) in adipocytes. *Diabetes*, **67**(2), 222–234.

901
902
903
904