Comparison of Transcriptional Activation by Corticosteroids of Human MR (Ile-180) and Human MR Haplotype (Ile180Val)

Yoshinao Katsu[1,2,*], Jiawen Zhang[2], Ya Ao[2], Michael E. Baker[3,4,*]

[1] Faculty of Science

Hokkaido University

Sapporo, Japan

[2] Graduate School of Life Science

Hokkaido University

Sapporo, Japan

[3] Division of Nephrology-Hypertension

Department of Medicine, 0693

University of California, San Diego

9500 Gilman Drive

La Jolla, CA 92093-0693

Center for Academic Research and Training in Anthropogeny (CARTA) [4]

University of California, San Diego

La Jolla, CA 92093

*Correspondence to

Y. Katsu; E-mail: ykatsu@sci.hokudai.ac.jp (YK)

M. E. Baker; E-mail: mbaker@health.ucsd.edu (MEB)

Abstract While the classical function of human mineralocorticoid receptor (MR) is to regulate sodium and potassium homeostasis through aldosterone activation of the kidney MR, the MR

also is highly expressed in the brain, where the MR is activated by cortisol in response to stress. Here, we report the half-maximal response (EC50) and fold-activation by cortisol, aldosterone and other corticosteroids of human MR rs5522, a haplotype containing valine at codon 180 instead of isoleucine found in the wild-type MR (Ile-180). MR rs5522 (Val-180) has been studied for its actions in the human brain involving coping with stress and depression. We compared the EC50 and fold-activation by corticosteroids of MR rs5522 and wild-type MR transfected into HEK293 cells with either the TAT3 promoter or the MMTV promoter. Parallel studies investigated the binding of MR antagonists, spironolactone and progesterone, to MR rs5522. In HEK293 cells with the MMTV promotor, MR rs5522 had a slightly higher EC50 compared to wild-type MR and a similar level of fold-activation for all corticosteroids. In contrast, in HEK293 cells with the TAT3 promoter, MR 5522 had a higher EC50 (lower affinity) and higher fold-activation for cortisol compared to wild-type MR (Ile-180), while compared to wild-type MR, the EC50s of MR rs5522 for aldosterone and corticosterone were slightly lower and fold-activation was higher. Spironolactone and progesterone had similar antagonist activity for MR rs5522 and MR (Ile-180) in the presence of MMTV and TAT3 promoters in HEK293 cells.

Key words: mineralocorticoid receptor, MR (Ile180/Val180), MR haplotype rs5522, brain, stress, depression, cortisol

Running title: Transcriptional Activation by Corticosteroids of Human MR rs5522 (Ile180Val)

Introduction

The mineralocorticoid receptor (MR) is a ligand-activated transcription factor [1] that belongs to the nuclear receptor family along with other vertebrate steroid receptors: the glucocorticoid receptor (GR), progesterone receptor (PR), androgen receptor (AR) and estrogen receptor (ER) [1–6]. In humans and other terrestrial vertebrates the MR maintains electrolyte balance by regulating sodium and potassium transport in epithelial cells in the kidney and colon

52 [7–12]. However, the MR also has important physiological functions in many other tissues,
53 including brain, heart, skin and lungs [11,13–24].

54 Analysis of the human MR sequence by Arriza et al [1] revealed that, like other steroid
55 receptors, the human MR is composed of four modular functional domains: a large amino-
56 terminal domain (NTD) of about 600 amino acids, followed in the center by a DNA-binding
57 domain (DBD) of about 65 amino acids, followed by a small hinge domain of about 60 amino
58 acids that is connected to the ligand-binding domain of about 250 amino acids at the C-terminus,
59 where aldosterone, cortisol and other corticosteroids bind to activate transcription [1,2,25–27].

60 Arriza et al. [1] also reported that the MR sequence was closely related to the
61 glucocorticoid receptor (GR) sequence, consistent with evidence that some corticosteroids, such
62 as cortisol and corticosterone were ligands for both the MR and GR [13,15,20,26,28] and that
63 aldosterone, cortisol, corticosterone and 11-deoxycorticosterone have similar binding affinity for
64 human MR [1,20,28–30]. Activation of the MR by cortisol and corticosterone, two steroids that
65 are ligands for the GR [30–32], is consistent with the evolution of the GR and MR from a
66 common ancestral corticoid receptor (CR) in a cyclostome (jawless fish) that evolved about 550
67 million years ago at the base of the vertebrate line [26,33–39].

68 Humans contain three almost identical MR transcripts that differ at codons 180 and 241
69 in the NTD [40] (Fig 1). Human MR (Ile-180), cloned by Arriza et al [1], has been extensively
70 studied for transcriptional activation by aldosterone and other corticosteroids [1,28,29,31,32,40].
71 Transcriptional activation by aldosterone and other corticosteroids of human MR (Ile-180, Val-
72 241) and MR (Val-180, Val-241) has recently been reported [40]. Here, we focus on the
73 response to corticosteroids of another human MR, haplotype rs5522 (Val-180), which contains a
74 valine at codon 180 [41–44] (Figure 1). rs5522 (Val-180) has important physiological effects on
75 stress and depression in humans [43–47] making transcriptional activation by steroids of rs5522
76 of much interest.

77 Aldosterone [42,43] and cortisol [43,48] are transcriptional activators of MR rs5522.
78 However, EC50s and fold-activation by aldosterone, corticosterone and 11-deoxycorticosterone
79 for the rs5522 haplotype have not been reported. Activation of rs5522 by a corticosteroid other
80 than cortisol could provide clues to the unique physiological actions of rs5522 in the brain MR.

81 Nor has the binding of spironolactone and progesterone, two MR antagonists [49,50] to
82 rs5522been investigated.. To provide this information, we investigated the effect of
83 corticosteroids and of mineralocorticoid antagonists on transcriptional activation of rs5522 in
84 presence of two promotors: TAT3 [51,52] and MMTV [25,53]. We find that in HEK293 cells
85 with the MMTV promotor, each corticosteroid had a similar EC50 and fold-activation for rs5522
86 and wild-type MR (MR Ile-180). In contrast, in HEK293 cells with the TAT3 promoter,
87 compared to wild-type MR, rs5522 had a slightly lower EC50 for aldosterone and corticosterone
88 and a higher EC50 for cortisol and a similar EC50 for 11-deoxycorticosterone. However, fold-
89 activation for cortisol activation of rs5522 was 1.4-fold higher than cortisol activation of MR
90 (Ile-180), which is interesting because cortisol is the biological ligand for the brain MR
91 [17,18,20,22,23]. Compared to wild-type MR (Ile-180), rs5522 had a higher fold-activation of
92 1.26, 1.29 and 1.32, respectively, for aldosterone, corticosterone and 11-deoxycorticosterone.
93 We find that spironolactone and progesterone had similar antagonist activity for rs5522 and MR
94 (Ile-180) in the presence of the MMTV and TAT3 promoters in HEK293 cells. The absence of a
95 large difference in EC50 and/or fold-activation between rs5522 and (MR Ile-180) in their
96 response to corticosteroids suggests that other mechanisms are important in rs5522 regulation of
97 the response to stress and depression. These may include post-translational modification of the
98 NTD [24,54–58], formation of MR-GR heterodimers [59–64] and binding to specific promoters
99 [61,65].

100

101

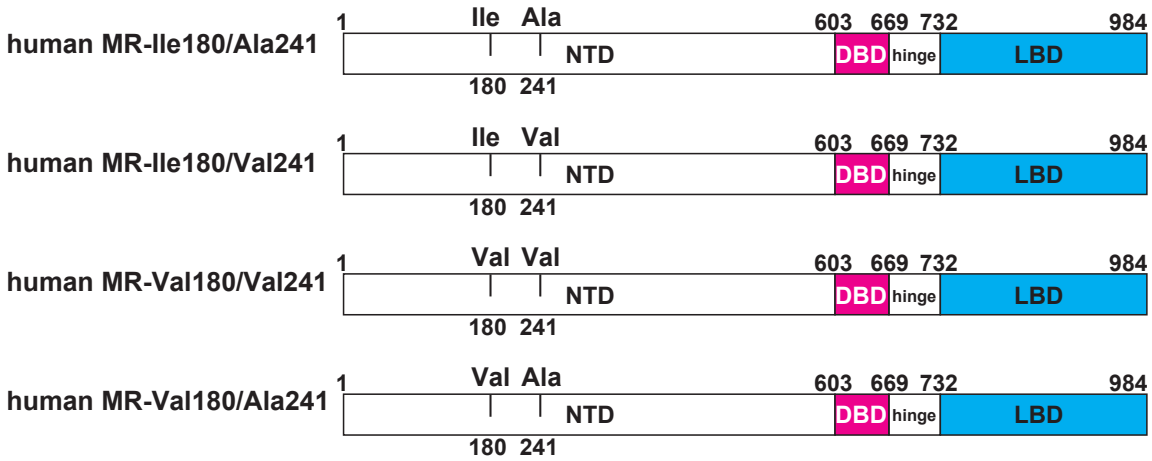


Fig 1. Human MR Genes.

There are three human MR genes with 984 amino acids in GenBank. These MRs contain either isoleucine-180, alanine-241 (Ile180/Ala241: accession AAA59571), isoleucine-180, valine-241 (Ile180/Val241: accession XP_054206038), or valine-180, valine-241 (Val180/Val241: accession NP_000892), in the NTD. The fourth MR isoform is rs5522, which contains valine-180 instead of isoleucine-180 (Val180/Ala241) [41–43].

Results

Corticosteroid-dependent activation of wild-type human MR (Ile-180) and rs5522 (MR Val-180)

We have used human MR (accession AAA59571) [1], which contains isoleucine at codon 180 for our studies of the human MR [29,40]. For this project, as described in the Methods Section, we constructed rs5522 from human MR (Ile-180, Ala-241). In Fig 2, we show the concentration dependence of transcriptional activation by corticosteroids of rs5522 and MR (Ile-180) transfected into HEK293 cells with either an MMTV or a TAT3 luciferase promoter. Luciferase levels were used to calculate an EC50 and fold-activation for each steroid (Table 1).

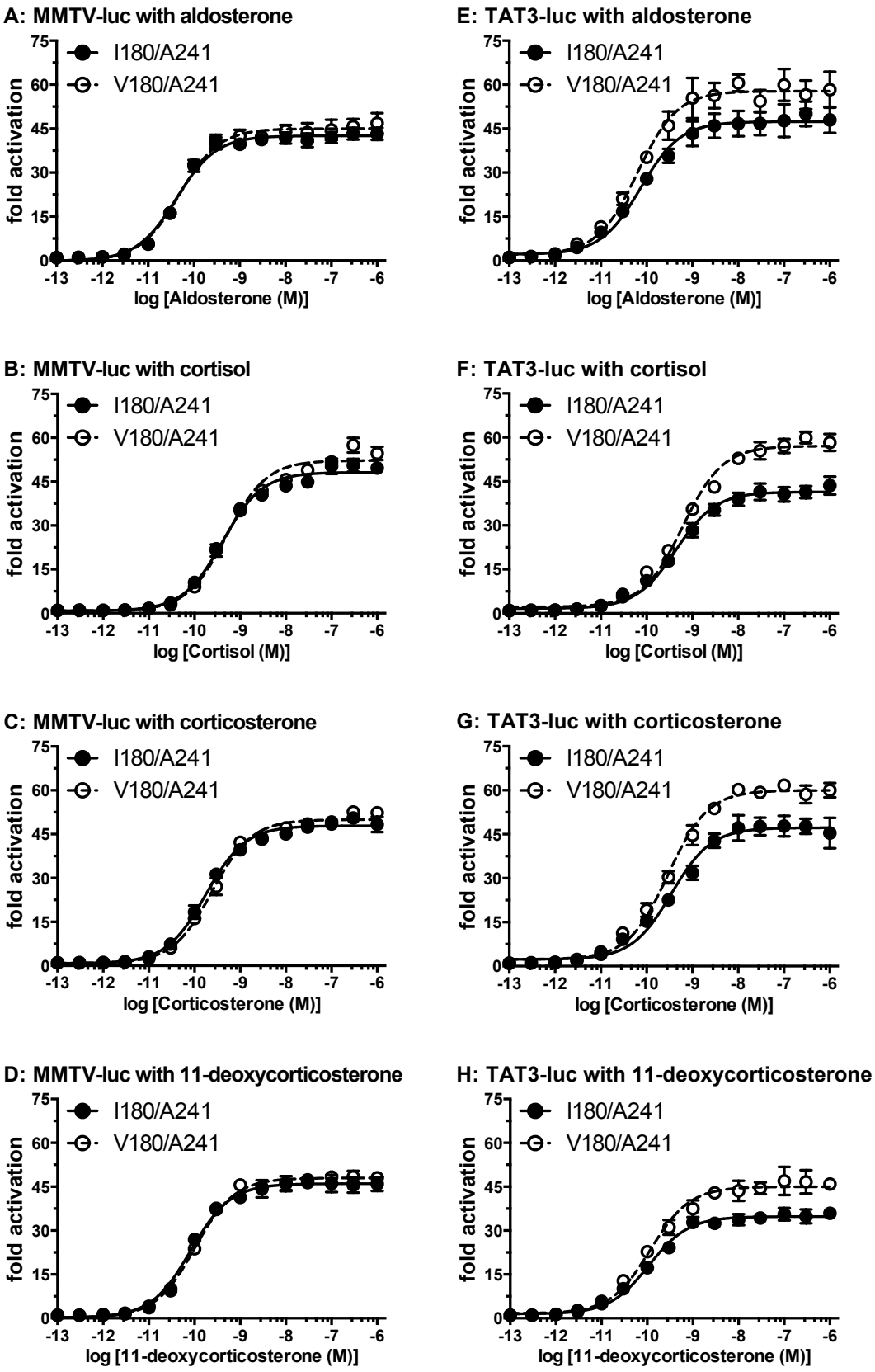
Fig 2 reveals that in HEK293 cells, transfected with the MMTV promoter, the EC50 and fold-activation values were similar for aldosterone, cortisol, corticosterone and 11-deoxycorticosterone. It is in the experiments with TAT3 promoter in HEK293 cells that we find differences between rs5522 and MR (Ile-180). As shown in Fig 2 and Table 1, in HEK293 cells

123 transfected with TAT3, and with either aldosterone or corticosterone had a slight decrease in
124 EC50 (stronger binding) for rs5522 compared to MR (Ile-180), while cortisol had a slight
125 increase in EC50 for rs5522 compared to MR (Ile-180). The EC50 of 11-deoxycorticosterone
126 was about the same value for rs5522 and MR (Ile-180).

127 Also as shown in Fig 2 and Table 1, in HEK293 cells transfected with TAT3, aldosterone
128 increased fold-activation by about 25% for rs5522 compared to MR (Ile-180). Cortisol, which
129 activates the MR in organs that lack 11β-hydroxysteroid dehydrogenase-type 2 [7,66–68],
130 increases fold activation by about 40% for rs5522 compared to MR (Ile-180). Corticosterone
131 and 11-deoxycorticosterone increased fold-activation by about 30% for rs5522 compared to MR
132 (Ile-180).

133

134


A: MMTV-luc with aldosterone
I180/A241
V180/A241
fold activation
log [Aldosterone (M)]
E: TAT3-luc with aldosterone
I180/A241
V180/A241
fold activation
log [Aldosterone (M)]
B: MMTV-luc with cortisol
I180/A241
V180/A241
fold activation
log [Cortisol (M)]
F: TAT3-luc with cortisol
I180/A241
V180/A241
fold activation
log [Cortisol (M)]
C: MMTV-luc with corticosterone
I180/A241
V180/A241
fold activation
log [Corticosterone (M)]
G: TAT3-luc with corticosterone
I180/A241
V180/A241
fold activation
log [Corticosterone (M)]
D: MMTV-luc with 11-deoxycorticosterone
I180/A241
V180/A241
fold activation
log [11-deoxycorticosterone (M)]
H: TAT3-luc with 11-deoxycorticosterone
I180/A241
V180/A241
fold activation
log [11-deoxycorticosterone (M)]

135

136 **Fig 2. Concentration-dependent transcriptional activation by corticosteroids of wild-type**
137 **human MR (Ile-180) and rs5522 (MR Val-180) in the presence of either the MMTV**
138 **promoter or the TAT3 promoter.**

139 Plasmids for wild-type MR (Ile-180) and (MR Val-180) were expressed in HEK293 cells with
140 either an MMTV-luciferase promoter (2A-2D) [25,53] or a TAT3-luciferase promoter (2E-2H)
141 [2,51,52]. Cells were treated with increasing concentrations of either aldosterone, cortisol, 11-
142 deoxycorticosterone, corticosterone, or vehicle alone (DMSO). Results are expressed as means
143 ± SEM, n=3. Y-axis indicates fold-activation compared to the activity of vector with vehicle
144 (DMSO) alone as 1. A. MR (Ile-180) and (MR Val-180) with aldosterone. B. MR (Ile-180) and
145 (MR Val-180) with cortisol. C. MR (Ile-180) and (MR Val-180) with corticosterone. D. MR
146 (Ile-180) and (MR Val-180) with 11-deoxycorticosterone. E. MR (Ile-180) and rs5522 (MR
147 Val-180) with aldosterone. F. MR (Ile-180) and rs5522 (MR Val-180) with cortisol. G. MR
148 (Ile-180) and (MR Val-180) with corticosterone. H. MR (Ile-180) and (MR Val-180) with 11-
149 deoxycorticosterone.

150

Table 1. Steroid Activation of Human MRs in HEK293 Cells with an MMTV or a TAT3 Promoter.

		MMTV-luc		**TAT3-luc**	
		I180/A241	V180/A241	I180/A241	V180/A241
Aldosterone	EC50 (nM)	0.042	0.048	0.074	0.063
	Fold-Activation (± SEM) *	41.9 (± 1.9)	44.5 (± 3.5)	47.7 (± 5.6)	59.9 (± 5.4)
	Ratio**	1	1.06	1	1.26
Cortisol	EC50 (nM)	0.41	0.51	0.43	0.62
	Fold-Activation (± SEM) *	50.3 (± 2.6)	51.7 (± 0.9)	40.6 (± 2.4)	57.1 (± 2.4)
	Ratio**	1	1.03	1	1.41
Corticosterone	EC50 (nM)	0.18	0.24	0.35	0.29
	Fold-Activation (± SEM) *	48.5 (± 1.7)	49.2 (± 0.9)	47.8 (± 3.5)	61.7 (± 0.06)
	Ratio**	1	1.01	1	1.29
11-deoxy-corticosterone	EC50 (nM)	0.083	0.1	0.11	0.12
	Fold-Activation (± SEM) *	46.1 (± 3.0)	48.3 (± 1.2)	35.6 (± 2.1)	47.0 (± 4.8)
	Ratio**	1	1.05	1	1.32

*Fold activation values were calculated based on the 100 nM values.

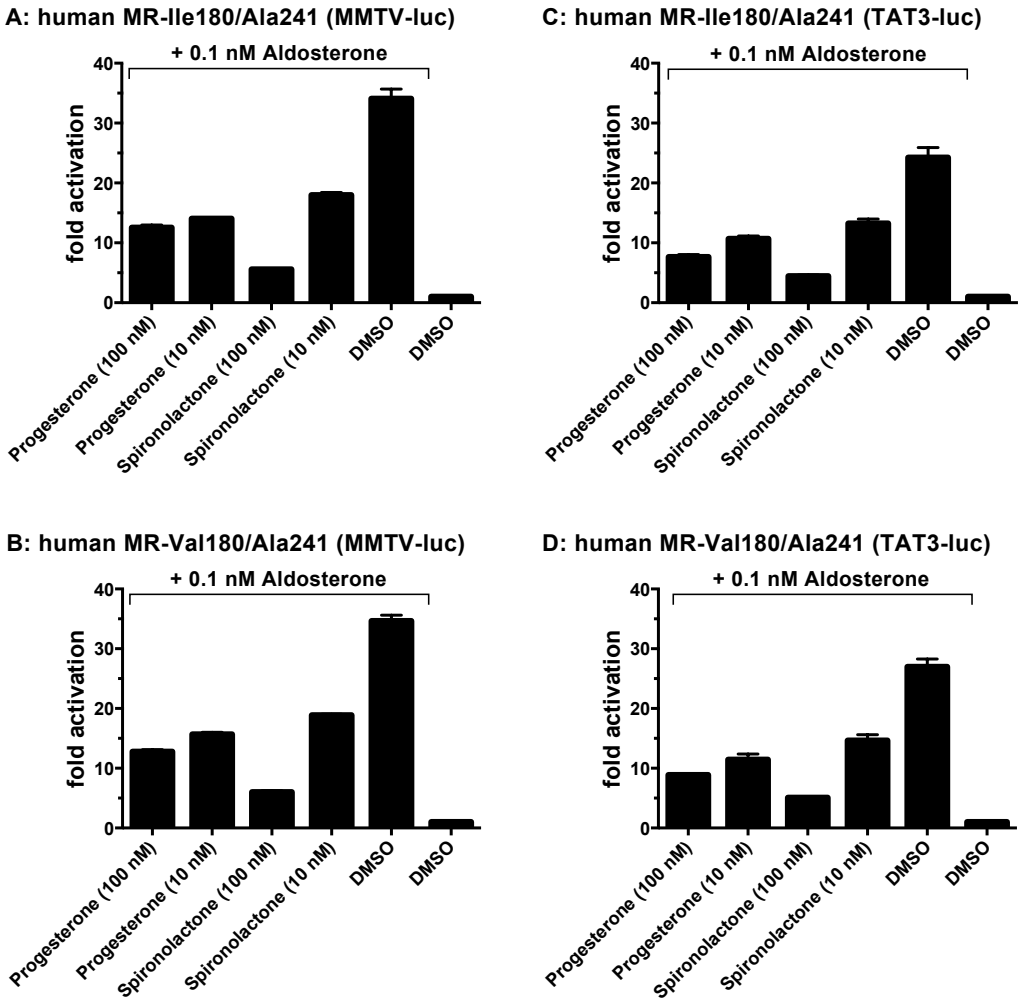
**Ratio values were calculated by dividing the fold-activation value of V180/A241 form by the value of I180/A241 form.

151

152

153 **Spironolactone and progesterone are antagonists for rs5522 and MR (Ile-180)**

154 To determine if spironolactone and progesterone are antagonists for rs5522, we added
155 either spironolactone or progesterone to HEK293 cells transfected with either human MR (Ile-
156 180) or rs5522 and either an MMTV promoter or a TAT3 promoter and incubated with 0.1 nM
157 aldosterone. Fig 3 shows that 10 nM spironolactone or 10 nM progesterone inhibit activation of
158 rs5522 and MR (Ile-180) by 0.1 nM aldosterone. We conclude that spironolactone and
159 progesterone are antagonists of rs5522 [49,50].



160

161

162 **Fig 3. Inhibition of Aldosterone Activation rs5522 and human MR (Ile-180, Ala-241) by**
163 **spironolactone and progesterone.**

164 **Figure Legend:** HEK293 cells were transfected with either human MR (Ile-180, Ala-241) or
165 rs5522 (Val-180, Ala-241) and either the MMTV promoter or the TAT3 promoter. Then these
166 cells were incubated with 0.1 nM aldosterone alone or with either 10 nM or 100 nM
167 spironolactone or progesterone for 24 hrs. HEK293 cells were harvested and processed for
168 luciferase.

169

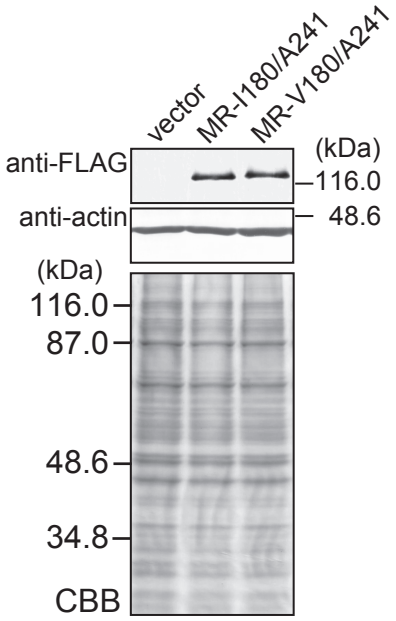
170 **FLAG-tagged expression of human MR (Ile-180) and rs5522**

171 To investigate the protein expression levels of FLAG-tagged MR (Ile-180) and rs5522
172 (Val-180), HEK293 cells transfected with each construct were collected and treated with SDS
173 sample buffer. Subsequently, FLAG-tagged proteins were subjected to SDS-PAGE on a 10%
174 polyacrylamide gel and transferred to an Immobilon membrane and detected using an antibody
175 against the FLAG tag. We detected a single band of human MR (Ile-180) and rs5522 (Fig 4),
176 which were used in the experiments depicted in Fig 2. This analysis of FLAG-tagged human
177 MR (Ile-180) and rs5522 (Val-180) confirms that there were similar protein expression levels of
178 these two MRs in our experiments.

179

180 **Fig 4 FLAG-tagged expression of human MR (Ile-180) rs5522 (Val-180).**

181 HEK293 cell lysates transfected with FLAG-tagged MR (Ile-180, Ala-241) and rs5522 (Val-180,
182 Ala-241) were treated with sample buffer and applied to a 10% SDS-polyacrylamide gel, and



183 then transferred onto a membrane. The expressed FLAG-tagged proteins were detected with
184 anti-FLAG antibody. The expression level of endogenous actin was measured using an actin
185 antibody. Upper panel: Western blot. Lower panel: Coomassie blue stain (CBB).

186

187 **Discussion**

188 The cloning of the human MR by Arriza et al [1] provided tools for investigating the
189 function of the MR in diverse tissues other than the distal tubule of the kidney, including the MR
190 in the hippocampus, which is receiving increasing attention as an MR-responsive organ
191 [19,20,22,23]. Indeed, the MR in the hippocampus is downregulated by extended stress and
192 depression [20,45,69,70], and MR synthesis is induced by anti-depressants. Increased activity of
193 the MR inhibits activity of the hypothalamic-pituitary-adrenal axis and promotes slow wave
194 cognition, reducing anxiety.

195 The MR rs5522 (Val-180), which is the focus of this report, is intriguing and important
196 because humans with MR (Val-180) have increased response to stress [22,23,43,46] despite the
197 presence of only a single amino acid difference between the rs5522, which contains valine at
198 codon 180, instead of isoleucine at codon 180 as found in wild-type MR [22,45,46,71]. Such a
199 profound physiological effect of a point mutation in the NTD, which is distant from the LBD and
200 DBD is unexpected. Moreover, valine-180 is not located in one of the transcriptional activation
201 domains in the NTD, which occur at residues 1-169 and 451-603 in the NTD of human MR
202 [72,73].

203 As a first step towards understanding the biological actions of the Ile180Val mutation in
204 human MR, we investigated transcriptional activation of MR (Val-180) by a panel of
205 corticosteroids including aldosterone and cortisol, for comparison with activation of wild-type
206 human MR [Figure 2, Table 1]. We studied transcriptional activation in the presence of either
207 the MMTV promoter or the TAT3 promoter. Interestingly, in cells with TAT3, but not in cells
208 with MMTV, there were differences in transcriptional activation of MR (Val-180) and wild-type
209 MR (Ile-180) by corticosteroids.

210 Among the corticosteroids, cortisol is of interest because in the human brain cortisol is
211 the physiological steroid for the MR because the circulating cortisol concentration is about 200-
212 fold higher compared to aldosterone, and11β-hydroxysteroid dehydrogenase-type 2, which can

213 inactivate cortisol [66–68], is absent in the hippocampus [66]. Cortisol had a higher EC50 for
214 MR (Val-180) than for wild-type MR in HEK293 cells transfected with TAT3, in agreement
215 with [43]. Interestingly, fold-activation of MR (Val-180)1 by cortisol was about 40% higher
216 than for cortisol activation of wild-type MR. Aldosterone had a slightly lower EC50 and 26%
217 higher fold-activation for MR (Val-180)1compared to wild type MR. Corticosterone and 11-
218 deoxycorticosterone had slightly lower EC50s and about 30% higher fold-activation for MR
219 (Val-180) than for wild-type MR. Spironolactone and progesterone, which inhibit wild-type MR
220 also inhibited aldosterone activation of MR (Val-180) [Figure 3].

221 The weaker EC50 and higher fold-activation of cortisol for MR (Val-180) by themselves
222 may be insufficient to support the profound biological response to stress and depression in
223 people with MR (Val-180) [20,45,46,69,70]. We intend to investigate other mechanism(s) that
224 may contribute to novel physiological activity of MR (Val-180). One possibility arises from the
225 different response to cortisol between MR (Val-180) and wild-type MR in the presence of either
226 TAT3 or MMTV [40,74], and the similar finding by DeRijk et al. [43] in the response to cortisol
227 of MR (Val-180) in cells with either TAT3 or MMTV promoters. It may be that other promoters
228 in the brain exert larger differences between MR (Val-180) and wild-type MR in transcriptional
229 activation by cortisol and other corticosteroids, which may be physiologically important.

230 Another possible mechanism is regulation of cortisol-mediated transcription of MR (Val-
231 180) by phosphorylation of one or more serine residues near codon 180 the NTD on the MR [54–
232 58]. Indeed, Shibata et al [54,57], Kino et al. [58] and Faresse et al. [75] have identified several
233 phosphorylation sites in the NTD of human MR. These sites include Ser-129, Ser-183, Ser-196,
234 Ser-227, Ser238, Ser-250 and Ser-263. Substitution of one or more serines in MR (Val-180)
235 with alanine or aspartic acid would determine whether phosphorylation is important in
236 corticosteroid activation of MR (Val-180).

237 We also will investigate the effect of the formation of heterodimers between MR (Val-
238 180) and the GR [59–64,76] on activation of MR (Val-180), which is relevant to MR activity in
239 the brain based on research by Mifsud and Ruel [61], who found that stress promotes MR-GR
240 heterodimers in the hippocampus. Promotion by stress of heterodimers between MR (Val-180)
241 and the GR may be important in the effects of MR (Val-180) on stress and depression.

242

243 **Materials & Methods**

244 **Construction of plasmid vectors**

245 Full-length mineralocorticoid receptor (MR) of human as registered in Genbank
246 (accession number: XP_054206038) was used as wild-type human MR. MR rs5522 was
247 constructed by the replacement of isoleucine-180 by valine using KOD-Plus-mutagenesis kit
248 (TOYOBO). The nucleic acid sequences of all constructs were verified by sequencing.

249

250 **Chemical reagents**

251 Cortisol, corticosterone, 11-deoxycorticosterone, aldosterone, progesterone and
252 spironolactone were purchased from Sigma-Aldrich. For reporter gene assays, all hormones
253 were dissolved in dimethyl-sulfoxide (DMSO); the final DMSO concentration in the culture
254 medium did not exceed 0.1%.

255

256 **Transactivation assays and statistical analyses**

257 Transfection and reporter assays were carried out in HEK293 cells, as described
258 previously [29]. HEK293 cells were seeded in 24-well plates at 5x104 cells/well in phenol-red
259 free Dulbecco's modified Eagle's medium supplemented with 10% charcoal-stripped fetal calf
260 serum (HyClone). After 24 hours, the cells were transfected with 100 ng of receptor gene,
261 reporter gene containing the *Photinus pyralis* luciferase gene and pRL-tk, as an internal control
262 to normalize for variation in transfection efficiency; pRL-tk contains the *Renilla reniformis*
263 luciferase gene with the herpes simplex virus thymidine kinase promoter. Each assay had a
264 similar number of cells, and assays were done with the same batch of cells in each experiment.
265 All experiments were performed in triplicate. Promoter activity was calculated as firefly (*P.*
266 *pyralis*)-lucifease activity/sea pansy (*R. reniformis*)-lucifease activity. The values shown are
267 mean ± SEM from three separate experiments, and dose-response data, which were used to
268 calculate the half maximal response (EC50) for each steroid, were analyzed using GraphPad
269 Prism.

270

271 **Antibodies and Western blotting of FLAG-tagged MR**

272 Primary antibodies for Western blotting were purchased and used at the indicated
273 dilutions: anti-FLAG (clone #1E6, FUJIFILM Wako Pure Chemical Corporation, 1:750), and
274 anti-betaActin (clone # 6D1, Medical & Biological Laboratories Co., LTD., 1:3000). Cells
275 transfected as described in "Transactivation assays and statistical analysis" were directly lysed
276 with sodium dodecyl sulfate (SDS) sample buffer and subjected to SDS-PAGE on a 10%
277 polyacrylamide gel and transferred to an Immobilon membrane. The membrane was blocked
278 with 10% skim milk overnight at 4 °C and then incubated with a primary antibody for 4 hours at
279 room temperature. The membrane was washed with TTBS (0.1% Tween-20 in Tris-buffered
280 saline) for at three times for 10 min and incubated with AP-conjugated secondary antibody
281 (COSMO BIO Co. Ltd.) for 1 hour at room temperature. After the final wash with TTBS, the
282 membrane was treated with NBT-BCIP solution). The gels were also stained with Coomassie
283 Blue.

284

285 **Funding:** This work was supported by Grants-in-Aid for Scientific Research from the Ministry
286 of Education, Culture, Sports, Science and Technology of Japan (23K05839) to Y.K., and the
287 Takeda Science Foundation to Y.K.

288

289 **Competing interests:** The authors have declared that no competing interests exist.

290

291 **Contributions**.

292 Yoshinao Katsu: Investigation, Conceptualization, Supervision, Formal Analysis, Writing –
293 original draft, Writing – review & editing.

294 Jiawen Zhang: Data curation, Investigation, Methodology.

295 Ya Ao: Data curation, Methodology.

296 Michael E. Baker: Conceptualization; Formal analysis, Supervision, Writing – original draft,
297 Writing – review & editing.

298

299 **References**

300 1. Arriza JL, Weinberger C, Cerelli G, et al. Cloning of human mineralocorticoid receptor
301 complementary DNA: structural and functional kinship with the glucocorticoid receptor.
302 Science. 1987;237(4812):268-275. doi:10.1126/science.3037703.

303 2. Evans RM. The steroid and thyroid hormone receptor superfamily. Science.
304 1988;240(4854):889-895. doi:10.1126/science.3283939.

305 3. Bridgham JT, Eick GN, Larroux C, et al. Protein evolution by molecular tinkering:
306 diversification of the nuclear receptor superfamily from a ligand-dependent ancestor. PLoS
307 Biol. 2010;8(10):e1000497. Published 2010 Oct 5. doi:10.1371/journal.pbio.1000497.

308 4. Baker ME, Nelson DR, Studer RA. Origin of the response to adrenal and sex steroids: Roles
309 of promiscuity and co-evolution of enzymes and steroid receptors. J Steroid Biochem Mol
310 Biol. 2015;151:12-24. doi:10.1016/j.jsbmb.2014.10.020.

311 5. Baker ME. Steroid receptors and vertebrate evolution. Mol Cell Endocrinol.
312 2019;496:110526. doi:10.1016/j.mce.2019.110526.

313 6. Evans RM, Mangelsdorf DJ. Nuclear Receptors, RXR, and the Big Bang. Cell. 2014 Mar
314 27;157(1):255-66. doi: 10.1016/j.cell.2014.03.012. PMID: 24679540; PMCID:
315 PMC4029515.

316 7. Rossier BC, Baker ME, Studer RA. Epithelial sodium transport and its control by
317 aldosterone: the story of our internal environment revisited. Physiol Rev. 2015;95(1):297-
318 340. doi:10.1152/physrev.00011.2014.

319 8. Hanukoglu I, Hanukoglu A. Epithelial sodium channel (ENaC) family: Phylogeny, structure-
320 function, tissue distribution, and associated inherited diseases. Gene. 2016;579(2):95-132.
321 doi:10.1016/j.gene.2015.12.061.

322 9. Lifton RP, Gharavi AG, Geller DS. Molecular mechanisms of human hypertension. Cell.
323 2001;104(4):545-556. doi:10.1016/s0092-8674(01)00241-0.

324 10. Shibata S. 30 YEARS OF THE MINERALOCORTICOID RECEPTOR: Mineralocorticoid
325 receptor and NaCl transport mechanisms in the renal distal nephron. J Endocrinol.
326 2017;234(1):T35-T47. doi:10.1530/JOE-16-0669.

327 11. Grossmann C, Almeida-Prieto B, Nolze A, Alvarez de la Rosa D. Structural and molecular
328 determinants of mineralocorticoid receptor signalling. Br J Pharmacol. 2021 Nov 22. doi:
329 10.1111/bph.15746. Epub ahead of print. PMID: 34811739.

330 12. Fernandes-Rosa FL, Boulkroun S, Fedlaoui B, Hureaux M, Travers-Allard S, Drossart T,
331 Favier J, Zennaro MC. New advances in endocrine hypertension: from genes to biomarkers.

332 Kidney Int. 2023 Mar;103(3):485-500. doi: 10.1016/j.kint.2022.12.021. Epub 2023 Jan 13.
333 PMID: 36646167.

334 13. Hawkins UA, Gomez-Sanchez EP, Gomez-Sanchez CM, Gomez-Sanchez CE. The
335 ubiquitous mineralocorticoid receptor: clinical implications. Curr Hypertens Rep.
336 2012;14(6):573-580. doi:10.1007/s11906-012-0297-0.

337 14. Jaisser F, Farman N. Emerging Roles of the Mineralocorticoid Receptor in Pathology:
338 Toward New Paradigms in Clinical Pharmacology. Pharmacol Rev. 2016;68(1):49-75.
339 doi:10.1124/pr.115.011106.

340 15. Funder J. 30 YEARS OF THE MINERALOCORTICOID RECEPTOR: Mineralocorticoid
341 receptor activation and specificity-conferring mechanisms: a brief history. J Endocrinol.
342 2017 Jul;234(1):T17-T21. doi: 10.1530/JOE-17-0119. Epub 2017 May 22. PMID:
343 28533421.

344 16. Gomez-Sanchez EP. Brain mineralocorticoid receptors in cognition and cardiovascular
345 homeostasis. Steroids. 2014 Dec;91:20-31. doi: 10.1016/j.steroids.2014.08.014. PMID:
346 25173821; PMCID: PMC4302001.

347 17. De Kloet ER, Vreugdenhil E, Oitzl MS, Joëls M. Brain corticosteroid receptor balance in
348 health and disease. Endocr Rev. 1998 Jun;19(3):269-301. doi: 10.1210/edrv.19.3.0331.
349 PMID: 9626555.

350 18. Arriza JL, Simerly RB, Swanson LW, Evans RM. The neuronal mineralocorticoid receptor
351 as a mediator of glucocorticoid response. Neuron. 1988 Nov;1(9):887-900. doi:
352 10.1016/0896-6273(88)90136-5. PMID: 2856104.

353 19. de Kloet ER, Joëls M. Brain mineralocorticoid receptor function in control of salt balance
354 and stress-adaptation. Physiol Behav. 2017 Sep 1;178:13-20. doi:
355 10.1016/j.physbeh.2016.12.045. Epub 2017 Jan 13. PMID: 28089704.

356 20. Paul SN, Wingenfeld K, Otte C, Meijer OC. Brain mineralocorticoid receptor in health and
357 disease: From molecular signalling to cognitive and emotional function. Br J Pharmacol.
358 2022 Jul;179(13):3205-3219. doi: 10.1111/bph.15835. Epub 2022 Apr 7. PMID: 35297038;
359 PMCID: PMC9323486.

360 21. Ibarrola J, Jaffe IZ. The Mineralocorticoid Receptor in the Vasculature: Friend or Foe? Annu
361 Rev Physiol. 2024 Feb 12;86:49-70. doi: 10.1146/annurev-physiol-042022-015223. Epub
362 2023 Oct 3. PMID: 37788489.

363 22. de Kloet ER. Brain mineralocorticoid and glucocorticoid receptor balance in neuroendocrine
364 regulation and stress-related psychiatric etiopathologies. Curr Opin Endocr Metab Res.
365 2022 Jun;24:100352. doi: 10.1016/j.coemr.2022.100352. PMID: 38037568; PMCID:
366 PMC10687720.

367 23. Gaudenzi C, Mifsud KR, Reul JMHM. Insights into isoform-specific mineralocorticoid
368 receptor action in the hippocampus. J Endocrinol. 2023 Jul 12;258(2):e220293. doi:
369 10.1530/JOE-22-0293. PMID: 37235709.

370 24. Yang J, Fuller PJ. Interactions of the mineralocorticoid receptor--within and without. Mol
371 Cell Endocrinol. 2012 Mar 24;350(2):196-205. doi: 10.1016/j.mce.2011.07.001. Epub 2011
372 Jul 18. PMID: 21784126.

373 25. Beato M, Arnemann J, Chalepakis G, Slater E, Willmann T. Gene regulation by steroid
374 hormones. J Steroid Biochem. 1987;27(1-3):9-14. doi: 10.1016/0022-4731(87)90288-3.
375 PMID: 2826895.

376 26. Baker ME, Katsu Y. 30 YEARS OF THE MINERALOCORTICOID RECEPTOR:
377 Evolution of the mineralocorticoid receptor: sequence, structure and function. J Endocrinol.
378 2017;234(1):T1-T16. doi:10.1530/JOE-16-0661.

379 27. Rogerson FM, Fuller PJ. Interdomain interactions in the mineralocorticoid receptor. Mol Cell
380 Endocrinol. 2003 Feb 28;200(1-2):45-55. doi: 10.1016/s0303-7207(02)00413-6. PMID:
381 12644298.

382 28. Rupprecht R, Arriza JL, Spengler D, et al. Transactivation and synergistic properties of the
383 mineralocorticoid receptor: relationship to the glucocorticoid receptor. Mol Endocrinol.
384 1993;7(4):597-603. doi:10.1210/mend.7.4.8388999.

385 29. Katsu Y, Oka K, Baker ME. Evolution of human, chicken, alligator, frog, and zebrafish
386 mineralocorticoid receptors: Allosteric influence on steroid specificity. Sci Signal. 2018 Jul
387 3;11(537):eaao1520. doi: 10.1126/scisignal.aao1520. PMID: 29970600.

388 30. Funder JW. Glucocorticoid and mineralocorticoid receptors: biology and clinical relevance.
389 Annu Rev Med. 1997;48:231-40. doi: 10.1146/annurev.med.48.1.231. PMID: 9046958.

390 31. Hellal-Levy C, Couette B, Fagart J, Souque A, Gomez-Sanchez C, Rafestin-Oblin M.
391 Specific hydroxylations determine selective corticosteroid recognition by human
392 glucocorticoid and mineralocorticoid receptors. FEBS Lett. 1999;464(1-2):9-13.
393 doi:10.1016/s0014-5793(99)01667-1.

394 32. Grossmann C, Scholz T, Rochel M, Bumke-Vogt C, Oelkers W, Pfeiffer AF, Diederich S,
395 Bahr V. Transactivation via the human glucocorticoid and mineralocorticoid receptor by
396 therapeutically used steroids in CV-1 cells: a comparison of their glucocorticoid and
397 mineralocorticoid properties. Eur J Endocrinol. 2004 Sep;151(3):397-406. doi:
398 10.1530/eje.0.1510397. PMID: 15362971.

399 33. Shimeld SM, Donoghue PC. Evolutionary crossroads in developmental biology: cyclostomes
400 (lamprey and hagfish). Development. 2012 Jun;139(12):2091-9. doi: 10.1242/dev.074716.
401 PMID: 22619386.

402 34. Close DA, Yun SS, McCormick SD, Wildbill AJ, Li W. 11-deoxycortisol is a corticosteroid
403 hormone in the lamprey. Proc Natl Acad Sci U S A. 2010 Aug 3;107(31):13942-7. doi:
404 10.1073/pnas.0914026107. Epub 2010 Jul 19. PMID: 20643930; PMCID: PMC2922276.

405 35. Thornton JW. Evolution of vertebrate steroid receptors from an ancestral estrogen receptor
406 by ligand exploitation and serial genome expansions. Proc Natl Acad Sci U S A. 2001 May
407 8;98(10):5671-6. doi: 10.1073/pnas.091553298. Epub 2001 May 1. PMID: 11331759;
408 PMCID: PMC33271.

409 36. Smith JJ, Kuraku S, Holt C, Sauka-Spengler T, Jiang N, Campbell MS, Yandell MD,
410 Manousaki T, Meyer A, Bloom OE, Morgan JR, Buxbaum JD, Sachidanandam R, Sims C,
411 Garruss AS, Cook M, Krumlauf R, Wiedemann LM, Sower SA, Decatur WA, Hall JA,
412 Amemiya CT, Saha NR, Buckley KM, Rast JP, Das S, Hirano M, McCurley N, Guo P,
413 Rohner N, Tabin CJ, Piccinelli P, Elgar G, Ruffier M, Aken BL, Searle SM, Muffato M,
414 Pignatelli M, Herrero J, Jones M, Brown CT, Chung-Davidson YW, Nanlohy KG, Libants
415 SV, Yeh CY, McCauley DW, Langeland JA, Pancer Z, Fritzsch B, de Jong PJ, Zhu B,
416 Fulton LL, Theising B, Flicek P, Bronner ME, Warren WC, Clifton SW, Wilson RK, Li W.
417 Sequencing of the sea lamprey (Petromyzon marinus) genome provides insights into
418 vertebrate evolution. Nat Genet. 2013 Apr;45(4):415-21, 421e1-2. doi: 10.1038/ng.2568.
419 Epub 2013 Feb 24. PMID: 23435085; PMCID: PMC3709584.

420 37. York JR, McCauley DW. The origin and evolution of vertebrate neural crest cells. Open
421 Biol. 2020 Jan;10(1):190285. doi: 10.1098/rsob.190285. Epub 2020 Jan 29. PMID:
422 31992146; PMCID: PMC7014683.

423 38. Kuratani S. Evo-devo studies of cyclostomes and the origin and evolution of jawed
424 vertebrates. Curr Top Dev Biol. 2021;141:207-239. doi: 10.1016/bs.ctdb.2020.11.011.
425 Epub 2020 Dec 13. PMID: 33602489.

426 39. Janvier P. microRNAs revive old views about jawless vertebrate divergence and evolution.
427 Proc Natl Acad Sci U S A. 2010 Nov 9;107(45):19137-8. doi: 10.1073/pnas.1014583107.
428 Epub 2010 Nov 1. PMID: 21041649; PMCID: PMC2984170.

429 40. Katsu Y, Zhang J, Baker ME. Lysine-Cysteine-Serine-Tryptophan inserted into the DNA-
430 binding domain of human mineralocorticoid receptor increases transcriptional activation by
431 aldosterone. J Steroid Biochem Mol Biol. 2024 Oct;243:106548. doi:
432 10.1016/j.jsbmb.2024.106548. Epub 2024 May 29. PMID: 38821293.

433 41. Arai K, Tsigos C, Suzuki Y, Irony I, Karl M, Listwak S, Chrousos GP. Physiological and
434 molecular aspects of mineralocorticoid receptor action in pseudohypoaldosteronism: a
435 responsiveness test and therapy. J Clin Endocrinol Metab. 1994 Oct;79(4):1019-23. doi:
436 10.1210/jcem.79.4.7962269. PMID: 7962269.

437 42. Arai K, Nakagomi Y, Iketani M, Shimura Y, Amemiya S, Ohyama K, Shibasaki T.
438 Functional polymorphisms in the mineralocorticoid receptor and amirolide-sensitive
439 sodium channel genes in a patient with sporadic pseudohypoaldosteronism. Hum Genet.
440 2003 Jan;112(1):91-7. doi: 10.1007/s00439-002-0855-7. Epub 2002 Oct 25. PMID:
441 12483305.

442 43. DeRijk RH, Wüst S, Meijer OC, Zennaro MC, Federenko IS, Hellhammer DH, Giacchetti G,
443 Vreugdenhil E, Zitman FG, de Kloet ER. A common polymorphism in the
444 mineralocorticoid receptor modulates stress responsiveness. J Clin Endocrinol Metab. 2006
445 Dec;91(12):5083-9. doi: 10.1210/jc.2006-0915. Epub 2006 Oct 3. PMID: 17018659.

446 44. Derijk RH, de Kloet ER. Corticosteroid receptor polymorphisms: determinants of
447 vulnerability and resilience. Eur J Pharmacol. 2008 Apr 7;583(2-3):303-11. doi:
448 10.1016/j.ejphar.2007.11.072. Epub 2008 Jan 30. PMID: 18321483.

449 45. de Kloet ER, Otte C, Kumsta R, Kok L, Hillegers MH, Hasselmann H, Kliegel D, Joëls M.
450 Stress and Depression: a Crucial Role of the Mineralocorticoid Receptor. J
451 Neuroendocrinol. 2016 Aug;28(8). doi: 10.1111/jne.12379. PMID: 26970338.

452 46. Bogdan R, Perlis RH, Fagerness J, Pizzagalli DA. The impact of mineralocorticoid receptor
453 ISO/VAL genotype (rs5522) and stress on reward learning. Genes Brain Behav. 2010
454 Aug;9(6):658-67. doi: 10.1111/j.1601-183X.2010.00600.x. Epub 2010 Jun 7. PMID:
455 20528958; PMCID: PMC2921022.

456 47. Kuningas M, de Rijk RH, Westendorp RG, Jolles J, Slagboom PE, van Heemst D. Mental
457 performance in old age dependent on cortisol and genetic variance in the mineralocorticoid
458 and glucocorticoid receptors. Neuropsychopharmacology. 2007 Jun;32(6):1295-301. doi:
459 10.1038/sj.npp.1301260. Epub 2006 Nov 29. PMID: 17133261.

460 48. van Leeuwen N, Bellingrath S, de Kloet ER, Zitman FG, DeRijk RH, Kudielka BM, Wüst S.
461 Human mineralocorticoid receptor (MR) gene haplotypes modulate MR expression and
462 transactivation: implication for the stress response. Psychoneuroendocrinology. 2011
463 Jun;36(5):699-709. doi: 10.1016/j.psyneuen.2010.10.003. Epub 2010 Nov 20. PMID:
464 21095064.

465 49. Geller DS, Farhi A, Pinkerton N, et al. Activating mineralocorticoid receptor mutation in
466 hypertension exacerbated by pregnancy. Science. 2000;289(5476):119-123.
467 doi:10.1126/science.289.5476.119.

468 50. Kolkhof P, Bärfacker L. 30 YEARS OF THE MINERALOCORTICOID RECEPTOR:
469 Mineralocorticoid receptor antagonists: 60 years of research and development. J
470 Endocrinol. 2017 Jul;234(1):T125-T140. doi: 10.1530/JOE-16-0600. PMID: 28634268;
471 PMCID: PMC5488394.

472 51. Iñiguez-Lluhí JA, Pearce D. A common motif within the negative regulatory regions of
473 multiple factors inhibits their transcriptional synergy. Mol Cell Biol. 2000
474 Aug;20(16):6040-50. doi: 10.1128/mcb.20.16.6040-6050.2000. PMID: 10913186; PMCID:
475 PMC86080.

476 52. Lombès M, Binart N, Oblin ME, Joulin V, Baulieu EE. Characterization of the interaction of
477 the human mineralocorticosteroid receptor with hormone response elements. Biochem J.
478 1993 Jun 1;292 (Pt 2)(Pt 2):577-83. doi: 10.1042/bj2920577. PMID: 8389140; PMCID:
479 PMC1134249.

480 53. Cato AC, Skroch P, Weinmann J, Butkeraitis P, Ponta H. DNA sequences outside the
481 receptor-binding sites differently modulate the responsiveness of the mouse mammary
482 tumour virus promoter to various steroid hormones. EMBO J. 1988 May;7(5):1403-10.
483 PMID: 2842149; PMCID: PMC458390.

484 54. Shibata S, Ishizawa K, Wang Q, Xu N, Fujita T, Uchida S, Lifton RP. ULK1 Phosphorylates
485 and Regulates Mineralocorticoid Receptor. Cell Rep. 2018 Jul 17;24(3):569-576. doi:
486 10.1016/j.celrep.2018.06.072. PMID: 30021155.

487 55. Faresse N. Post-translational modifications of the mineralocorticoid receptor: How to dress
488 the receptor according to the circumstances? J Steroid Biochem Mol Biol. 2014
489 Sep;143:334-42. doi: 10.1016/j.jsbmb.2014.04.015. Epub 2014 May 10. PMID: 24820770.

490 56. Gadasheva Y, Nolze A, Grossmann C. Posttranslational Modifications of the
491 Mineralocorticoid Receptor and Cardiovascular Aging. Front Mol Biosci. 2021 May
492 28;8:667990. doi: 10.3389/fmolb.2021.667990. PMID: 34124152; PMCID: PMC8193679.

493 57. Shibata S, Rinehart J, Zhang J, Moeckel G, Castañeda-Bueno M, Stiegler AL, Boggon TJ,
494 Gamba G, Lifton RP. Mineralocorticoid receptor phosphorylation regulates ligand binding
495 and renal response to volume depletion and hyperkalemia. Cell Metab. 2013 Nov
496 5;18(5):660-71. doi: 10.1016/j.cmet.2013.10.005. PMID: 24206662; PMCID:
497 PMC3909709.

498 58. Kino T, Jaffe H, Amin ND, Chakrabarti M, Zheng YL, Chrousos GP, Pant HC. Cyclin-
499 dependent kinase 5 modulates the transcriptional activity of the mineralocorticoid receptor
500 and regulates expression of brain-derived neurotrophic factor. Mol Endocrinol. 2010
501 May;24(5):941-52. doi: 10.1210/me.2009-0395. Epub 2010 Mar 31. PMID: 20357208;
502 PMCID: PMC2870940.

503 59. Liu W, Wang J, Sauter NK, Pearce D. Steroid receptor heterodimerization demonstrated in
504 vitro and in vivo. Proc Natl Acad Sci U S A. 1995 Dec 19;92(26):12480-4. doi:
505 10.1073/pnas.92.26.12480. PMID: 8618925; PMCID: PMC40381.

506 60. Trapp T, Holsboer F. Heterodimerization between mineralocorticoid and glucocorticoid
507 receptors increases the functional diversity of corticosteroid action. Trends Pharmacol Sci.
508 1996 Apr;17(4):145-9. doi: 10.1016/0165-6147(96)81590-2. PMID: 8984741.

509 61. Mifsud KR, Reul JM. Acute stress enhances heterodimerization and binding of corticosteroid
510 receptors at glucocorticoid target genes in the hippocampus. Proc Natl Acad Sci U S A.
511 2016 Oct 4;113(40):11336-11341. doi: 10.1073/pnas.1605246113. Epub 2016 Sep 21.
512 PMID: 27655894; PMCID: PMC5056104.

513 62. Pooley JR, Rivers CA, Kilcooley MT, Paul SN, Cavga AD, Kershaw YM, Muratcioglu S,
514 Gursoy A, Keskin O, Lightman SL. Beyond the heterodimer model for mineralocorticoid
515 and glucocorticoid receptor interactions in nuclei and at DNA. PLoS One. 2020 Jan
516 10;15(1):e0227520. doi: 10.1371/journal.pone.0227520. PMID: 31923266; PMCID:
517 PMC6953809.

518 63. Kiilerich P, Triqueneaux G, Christensen NM, et al. Interaction between the trout
519 mineralocorticoid and glucocorticoid receptors in vitro. J Mol Endocrinol. 2015;55(1):55-
520 68. doi:10.1530/JME-15-0002.

521 64. Alvarez de la Rosa D, Ramos-Hernández Z, Weller-Pérez J, Johnson TA, Hager GL. The
522 impact of mineralocorticoid and glucocorticoid receptor interaction on corticosteroid
523 transcriptional outcomes. Mol Cell Endocrinol. 2024 Dec 1;594:112389. doi:
524 10.1016/j.mce.2024.112389. Epub 2024 Oct 17. PMID: 39423940.

525 65. Meijsing SH, Pufall MA, So AY, Bates DL, Chen L, Yamamoto KR. DNA binding site
526 sequence directs glucocorticoid receptor structure and activity. Science. 2009 Apr
527 17;324(5925):407-10. doi: 10.1126/science.1164265. PMID: 19372434; PMCID:
528 PMC2777810.

529 66. Chapman K, Holmes M, Seckl J. 11β-hydroxysteroid dehydrogenases: intracellular gate-
530 keepers of tissue glucocorticoid action. Physiol Rev. 2013 Jul;93(3):1139-206. doi:
531 10.1152/physrev.00020.2012. PMID: 23899562; PMCID: PMC3962546.

532 67. Frey FJ, Odermatt A, Frey BM. Glucocorticoid-Mediated Mineralocorticoid Receptor
533 Activation and Hypertension. Curr Opin Nephrol Hypertens. 2004 Jul;13(4):451-8. Doi:
534 10.1097/01.Mnh.0000133976.32559.B0. PMID: 15199296.

535 68. Funder JW. Apparent mineralocorticoid excess. J Steroid Biochem Mol Biol. 2017
536 Jan;165(Pt A):151-153. doi: 10.1016/j.jsbmb.2016.03.010. Epub 2016 Mar 5. PMID:
537 26956190.

538 69. Klok MD, Alt SR, Irurzun Lafitte AJ, Turner JD, Lakke EA, Huitinga I, Muller CP, Zitman
539 FG, de Kloet ER, Derijk RH. Decreased expression of mineralocorticoid receptor mRNA
540 and its splice variants in postmortem brain regions of patients with major depressive
541 disorder. J Psychiatr Res. 2011 Jul;45(7):871-8. doi: 10.1016/j.jpsychires.2010.12.002.
542 Epub 2010 Dec 30. PMID: 21195417.

543 70. Medina A, Seasholtz AF, Sharma V, Burke S, Bunney W Jr, Myers RM, Schatzberg A, Akil
544 H, Watson SJ. Glucocorticoid and mineralocorticoid receptor expression in the human
545 hippocampus in major depressive disorder. J Psychiatr Res. 2013 Mar;47(3):307-14. doi:
546 10.1016/j.jpsychires.2012.11.002. Epub 2012 Dec 6. PMID: 23219281; PMCID:
547 PMC4248661.

548 71. Klok MD, Giltay EJ, Van der Does AJ, Geleijnse JM, Antypa N, Penninx BW, de Geus EJ,
549 Willemsen G, Boomsma DI, van Leeuwen N, Zitman FG, de Kloet ER, DeRijk RH. A
550 common and functional mineralocorticoid receptor haplotype enhances optimism and
551 protects against depression in females. Transl Psychiatry. 2011 Dec 13;1(12):e62. doi:
552 10.1038/tp.2011.59. PMID: 22832354; PMCID: PMC3309494.

553 72. Fuse H, Kitagawa H, Kato S. Characterization of transactivational property and coactivator
554 mediation of rat mineralocorticoid receptor activation function-1 (AF-1). Mol Endocrinol.
555 2000 Jun;14(6):889-99. doi: 10.1210/mend.14.6.0467. PMID: 10847590.

556 73. Fischer K, Kelly SM, Watt K, Price NC, McEwan IJ. Conformation of the mineralocorticoid
557 receptor N-terminal domain: evidence for induced and stable structure. Mol Endocrinol.
558 2010 Oct;24(10):1935-48. doi: 10.1210/me.2010-0005. Epub 2010 Aug 4. PMID:
559 20685853; PMCID: PMC5417395.

560 74. Katsu Y, Oana S, Lin X, Hyodo S, Baker ME. Aldosterone and dexamethasone activate
561 African lungfish mineralocorticoid receptor: Increased activation after removal of the
562 amino-terminal domain. J Steroid Biochem Mol Biol. 2022 Jan;215:106024. doi:
563 10.1016/j.jsbmb.2021.106024. Epub 2021 Nov 10. PMID: 34774724.

564 75. Faresse N, Vitagliano JJ, Staub O. Differential ubiquitylation of the mineralocorticoid
565 receptor is regulated by phosphorylation. FASEB J. 2012 Oct;26(10):4373-82. doi:
566 10.1096/fj.12-209924. Epub 2012 Jul 13. PMID: 22798426.

567 76. Johnson TA, Fettweis G, Wagh K, Ceacero-Heras D, Krishnamurthy M, Sánchez de Medina
568 F, Martínez-Augustin O, Upadhyaya A, Hager GL, Alvarez de la Rosa D. The
569 glucocorticoid receptor potentiates aldosterone-induced transcription by the
570 mineralocorticoid receptor. Proc Natl Acad Sci U S A. 2024 Nov 19;121(47):e2413737121.
571 doi: 10.1073/pnas.2413737121. Epub 2024 Nov 14. PMID: 39541347; PMCID:
572 PMC11588051.

573