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Overview of Aldosterone-related Genetic Syndromes and Recent Advances

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Abstract

Purpose of review: Primary aldosteronism is the most common form of secondary hypertension. Early diagnosis and treatment are key to cure of hypertension and prevention of cardiovascular complications. Recent genetic discoveries have improved our understanding on the pathophysiology of aldosterone production and triggered the development of new diagnostic procedures and targeted treatments for PA.

Recent findings: Different inherited genetic abnormalities distinguish specific forms of familial hyperaldosteronism. Somatic mutations are found not only in aldosterone producing adenoma leading to primary aldosteronism, but also in aldosterone producing cell clusters of normal and micronodules from image-negative adrenal glands. Genetic knowledge has allowed the discovery of surrogate biomarkers and specific pharmacological inhibitors. Aging appears to be associated with dysregulated and relatively autonomous aldosterone production.

Summary: New biochemical markers and pharmacological approaches may allow pre-operative identification of somatic mutation carriers and use of targeted treatments.

Keywords: hypertension, adrenal, primary aldosteronism; ion channels; calcium

Introduction

1
2 Aldosterone-related genetic syndromes encompass diseases related to abnormal aldosterone
3 production or abnormal aldosterone effects in target organs. Given the important role of
4 aldosterone in regulating sodium and potassium balance and blood pressure in the kidney,
5 their main clinical manifestations are arterial hypertension or salt wasting and dehydration.
6 Different diseases affect aldosterone production in the adrenal cortex. There exist several
7 Mendelian forms of hyperaldosteronism, termed familial hyperaldosteronism (FH) type I to
8 IV, which share most of the phenotype but show some peculiarities. They are due to gain of
9 function mutations in ion channels and pumps, leading to increased and autonomous
10 aldosterone production. Somatic mutations in the same genes leading to FH have been
11 identified in aldosterone producing adenoma (APA) leading to primary aldosteronism (PA).
12 Increased aldosterone production can also be due to monogenic forms of adrenal insufficiency
13 affecting cortisol production, while loss-of-function mutations in *CYP11B2*, coding for
14 aldosterone synthase, lead to aldosterone deficiency, also called corticosterone methyl oxidase
15 deficiency; those syndromes will be treated elsewhere in this section. On the other hand,
16 different monogenic disorders affect aldosterone action in target tissues. They include a rare
17 disease of aldosterone resistance in peripheral tissues named pseudohypoaldosteronism type 1
18 as well as excessive mineralocorticoid action due to different mechanisms either directly or
19 indirectly related to aldosterone. In this review we will focus on primary aldosteronism as
20 genetic disease strictu sensu related to aldosterone biosynthesis in the adrenal gland and then
21 describe some emerging concepts related to pathophysiological aldosterone production.

22 (Figure 1)

23

24 **The genetics of primary aldosteronism**

25 PA is the most frequent form of secondary arterial hypertension. It is due to aldosterone
26 production from the adrenal cortex relatively autonomous from the renin-angiotensin system,
27 due in most cases to APA or bilateral adrenal hyperplasia. Its prevalence is estimated to be
28 around 4% of all hypertensive patients seen in primary care (1), data recently confirmed in a
29 large prospective study (2), and 10% in patients referred to tertiary care centers (1). Starting
30 from 2011, important progress has been made in our understanding of the genetic mechanisms
31 involved in PA. Currently, four Mendelian forms of PA, transmitted as autosomal dominant
32 traits, have been identified (these are reviewed in detail in (3, 4)). FH-I, also known as
33 glucocorticoid remediable aldosteronism (5), is due to the formation of a chimeric gene
34 between the highly homologous genes *CYP11B1*, coding for 11 β -hydroxylase, and *CYP11B2*,
35 coding for aldosterone synthase (6), leading to ACTH-dependent aldosterone production.
36 Mutations in *KCNJ5* have been identified in patients with FH-III, a severe form of familial
37 hyperaldosteronism associated to massive bilateral hyperplasia (7), but in some cases
38 presenting as a mild form of the disease without adrenal abnormalities (3). Germline
39 heterozygous mutations in *CACNA1D* (encoding the L-type calcium channel Cav1.3 subunit
40 α -1D) have been identified in patients with PA, seizures and neurological abnormalities, a
41 rare disease called PASNA (8). Finally, mutations in *CACNA1H* (encoding the pore-forming
42 α 1 subunit of the T-type voltage-dependent calcium channel Cav3.2) have recently been
43 reported as a cause of a new form of inherited PA, called FH-IV, associated in some cases
44 with developmental disorder (9, 10). In addition, germline mutations in the same gene have
45 been associated with mild forms of PA in patients diagnosed as FH-II (10). FH-II is
46 indistinguishable from apparently non-familial PA and only diagnosed on the basis of two or
47 more affected individuals in the same family (11). Despite linkage to 7p22 in one family (12),
48 no causative locus has been identified so far. Given that germline *KCNJ5* and *CACNA1H*
49 mutations have been identified in patients diagnosed as FH-II, and that the clinical phenotype

50 of FH-II is heterogeneous, being associated to APA as well as bilateral adrenal hyperplasia, it
51 is reasonable to suspect that the disease may encompass different genetic syndromes.

52 In addition to germline variants, somatic mutations in *KCNJ5* (7) and *CACNAID* (8, 13), but
53 also ATPases (*ATP1A1* and *ATP2B3* (13, 14) have been identified in APA. Mutations in all
54 these genes affect aldosterone production by altering pathways involved in maintaining
55 intracellular ionic homeostasis and cell membrane potential, ultimately leading to increased
56 calcium signalling. In particular, mutations in *KCNJ5*, coding for the potassium channel
57 GIRK4, affect the selectivity filter of the channel and lead to increased sodium permeability
58 and sodium influx, cell membrane depolarization and opening of voltage-gated calcium
59 channels, followed by increased intracellular calcium concentrations and activation of calcium
60 signalling, the main trigger for aldosterone production (15). Similarly, mutations in *ATP1A1*,
61 encoding the $\alpha 1$ subunit of the Na^+ , K^+ -ATPase lead to loss of pump activity and inward
62 sodium or proton leak, which were suggested to depolarize the cell membrane and increase
63 aldosterone production through activation of calcium signaling (13, 14). More recently,
64 experiments performed in adrenocortical NCI-H295R cells failed to demonstrate
65 modifications of intracellular calcium concentrations in the presence of mutant Na^+ , K^+ -
66 ATPase $\alpha 1$ subunits and rather suggest a mechanism involving intracellular acidification (16).

67 The pathogenic mechanisms involved in stimulating aldosterone production in the presence of
68 *ATP1A1* mutations may therefore result from alterations in multiple pathways, possibly in
69 relation with the multiple roles of the Na^+ , K^+ -ATPase as a pump but also a signal transducing
70 platform (17). Mutations in *CACNAID* and *CACNAIH* directly increase intracellular calcium
71 concentrations, by modifying voltage-sensitivity and other functional properties of these
72 voltage-gated calcium channels (9, 10). Similarly, *ATP2B3* mutations affecting the plasma
73 membrane calcium-transporting ATPase 3 (PMCA3) lead to increased intracellular calcium
74 concentrations due to loss of the physiological pump function leading to reduced calcium

75 export (18). The mutations are also associated with severely depolarized membrane potential
76 of primary adenoma cells (14), which is explained by a pathological sodium leak associated
77 with or conducted by the mutant PMCA3 (18). This may be a consequence of the location of
78 the majority of PMCA3 mutations, which lie next to a “PEGL” motif in transmembrane helix
79 4, a region involved in calcium binding and ion gating. Disturbed intracellular ionic
80 homeostasis is suggested to cause plasma membrane depolarization, leading to opening of
81 voltage-gated Ca^{2+} channels and increased Ca^{2+} influx driving increased *CYP11B2* expression
82 and aldosterone biosynthesis. Finally, somatic mutations in *CTNNB1*, encoding β -catenin
83 have been described in 2-5% of APA (19, 20) and mutations in *PRKACA* (coding for the
84 cAMP-dependent protein kinase catalytic subunit alpha) in rare cases (21). Mutations in those
85 genes have previously been involved in cortisol producing adenoma and adrenocortical
86 cancer; the mechanisms whereby the same mutation leads to different hormonal phenotypes
87 remain yet to be established ((15), see below).

88

89 **Clinical implications of mutation detection in PA**

90 Mutation detection in familial forms of PA is routinely performed in some genetic
91 laboratories and is useful for disease screening in affected families, early diagnosis and
92 targeted treatment. In patients with APA, the challenge is represented by the detection of
93 somatic mutations which are absent in DNA extracted from peripheral blood cells.
94 Correlations of any biological feature associated with a particular mutation would allow to
95 identify mutation carriers who could be suitable candidates for adrenal vein sampling and
96 eventually targeted treatments (22, 23). The most frequent somatic event in APA are *KCNJ5*
97 mutations whose frequency ranges from 13-77%, with higher frequency in East Asian
98 populations ((15) and references therein). *CACNA1D* mutations are found in up to 10% of
99 cases, followed by *ATP1A1* and *ATP2B3* mutations. A meta-analysis of 13 studies

100 investigating a total of 1636 patients confirmed previous results showing that *KCNJ5*
101 mutations are more frequent in women and associated with younger age, larger tumor size and
102 higher plasma aldosterone levels (24). Genotype-specific steroid profiles have been recently
103 associated with APA (25). By profiling 15 steroids in adrenal venous and peripheral plasma
104 samples from 79 patients by liquid chromatography-tandem mass spectrometry, Williams et al
105 identified a 7-steroid fingerprint (including aldosterone, 18-oxocortisol, 18-hydroxycortisol,
106 21-deoxycortisol, corticosterone, 11-deoxycorticosterone, and cortisol) in peripheral vein
107 plasma able to correctly classify 92% of the APA according to genotype (25). In addition,
108 specific steroid features were identified, in particular the presence of significantly higher
109 hybrid steroids 18-hydroxycortisol and 18-oxocortisol in patients with APA due to *KCNJ5*
110 mutations. The usefulness of this type of screening is related to the possibility of selecting
111 patients for adrenal vein sampling, but also for developing targeted treatments for specific
112 mutation carriers. Initial studies of mutant *KCNJ5* channels showed that their pharmacology
113 was different from that of wild type channels (22). In particular, *KCNJ5* mutants were
114 blocked by calcium-channel blockers, such as verapamil, and Na-channel blockers, such as
115 amiloride. At high therapeutic doses (3 x 120 mg/d) plasma concentrations of verapamil could
116 be sufficient to block the most sensitive *KCNJ5* mutant p.Leu168Arg (22). More recently,
117 Scholl et al applied a high-throughput screen based on the rescue of cell lethality induced by
118 *KCNJ5* mutations. This strategy identified a series of macrolide antibiotics, including
119 roxithromycin, that potently inhibit the mutant channels (IC₅₀ of 0.22 μM for the *KCNJ5*
120 p.Gly151Arg mutant and 0.69 on the p.Leu168Arg) as well as *CYP11B2* expression and
121 aldosterone production in adrenocortical HAC15 cells (23). The authors suggest that
122 macrolides could be useful in screening tests aimed at identifying carriers of *KCNJ5*
123 mutations, which together with peculiar imaging characteristics of these tumors (larger size,
124 lower pre-contrast Hounsfield units on computed tomography compared with tumors carrying

125 mutations in other genes (19)) could guide subtype diagnosis for surgery. Those compounds
126 could also be used as targeted treatments in patients who are not candidates for surgery, given
127 their long pharmacological history and safety. In a proof-of-concept Study, Caroccia et al
128 tested the effects of clarithromycin on aldosterone biosynthesis in primary cell lines obtained
129 from APA tissues with or without the two most common *KCNJ5* mutations, p.Gly151Arg and
130 p.Leu168Arg (26). Remarkably, clarithromycin inhibited *CYP11B2* gene expression and
131 aldosterone production in a dose-dependent manner. Further studies are warranted to show
132 whether these effects can be translated to patients and whether macrolides or other
133 compounds can be used to identify and treat patients with PA due to APA with *KCNJ5*
134 mutations or patients with FH-III otherwise requiring bilateral adrenalectomy to control blood
135 pressure (27).

136

137 **How do APA develop?**

138 While the role of somatic mutations in regulating aldosterone biosynthesis has been clearly
139 established, the origin of APA is still a matter of debate. Different studies have been
140 published in the last few years leading to the emergence of two hypotheses for APA
141 development, the “two hit model” and the “APCC model”.

142 The two-hit model supports the idea of the occurrence of two consecutive events leading to
143 APA, a first one driving abnormal cell proliferation followed by a second one driving specific
144 hormonal secretion. Adrenal cortex remodelling, reduced vascularization and ZG hyperplasia
145 are major features of APA development (28, 29). In many cases, the resected adrenal gland
146 harbours not only one adenoma but other micro- or macronodules, some of them expressing
147 aldosterone synthase (28, 30, 31). Investigation of genetic abnormalities in multinodular
148 adrenals revealed the presence of different somatic APA driver mutations in different nodules
149 from the same adrenal (31, 32), strongly suggesting that mutations are a second event

150 occurring in a previously remodelled adrenal cortex. In many cases, APA have been described
151 to be heterogeneous with aldosterone synthase expressed only in some parts of the tumour
152 (33, 34); somatic mutations appear to be present only in the aldosterone synthase positive
153 region of the APA (33). The recent description of a patient with PA harbouring a germline
154 heterozygous *APC* gene mutation associated, in the resected multinodular adrenal, to biallelic
155 *APC* inactivation due to a loss of heterozygosity in all nodules, but a somatic *KCNJ5*
156 mutation only in the aldosterone synthase expressing nodule further supports the two-hit
157 model for APA development (35). *APC* is part of the WNT/ β -catenin pathway which plays a
158 major role in adrenal gland development and tumorigenesis (36). In APA, somatic mutations
159 in *CTNNB1*, encoding β -catenin, are present in a subset of APA (19, 20) and the WNT/ β -
160 catenin pathway is activated in two thirds of cases, independently of the mutation status (37,
161 38). This pathway may therefore constitute the first event leading to abnormal cell
162 proliferation and adenoma formation. Other signalling pathways and transcriptional cascades
163 may also be involved and create a propitious environment for the occurrence of mutations in
164 one of the APA driver genes.

165 On the other hand, the APCC model supports the idea that occurrence, in ZG cells, of a
166 somatic mutation in one of the APA driver genes leads to the formation of aldosterone-
167 producing cell clusters (APCC), followed, through the development of APCC-to-APA
168 translational lesions (pAATL), to APA formation (39). Next generation sequencing performed
169 on formalin-fixed, paraffin-embedded tissue DNA identified somatic mutations in some of the
170 known APA driver genes, i.e. *CACNAID* and *ATPIA1* in 8 of 23 APCC from normal adrenals
171 from kidney donors, most frequently affecting *CACNAID*. Interestingly, no mutation in the
172 *KCNJ5* gene, the most frequent alteration observed in APA, has been reported in APCC (39,
173 40). The recent description of pAATL consisting of a subcapsular APCC-like structure
174 (APCC-like) and an inner-APA-like structure (mAPA-like) without well-defined histological

175 border has suggested a possible APCC to APA transition (41). These specific lesions were
176 first described in adrenals from two patients with unilateral multiple adrenocortical
177 micronodules. To determine whether they could constitute intermediate structures between
178 APCC and APA, search for mutations in APA driver genes was performed in both APCC and
179 mAPA structures. In the first case a *KCNJ5* mutation was identified in frozen tissue from a
180 suspected aldosterone producing nodule. A different *KCNJ5* mutation resulting in the same
181 amino acid change was found in the mAPA-like part of a first pAATL, but not in its
182 neighbouring APCC-like structure, indicating that the aldosterone producing nodule and the
183 mAPA had different origins. The fact that only the mAPA portion had the mutation suggested
184 that the mutation may have led to a transdifferentiation of the cells into a mAPA like
185 phenotype. In a second pAATL, an *ATP1A1* mutation was found in the entire pAATL
186 suggesting a clonal origin of the entire structure. Histologically, the adrenal from the second
187 case revealed no major abnormalities except for the presence of a pAATL (42). In a recent
188 report of 4 additional cases, the same authors identified mutations in *KCNJ5* preferentially
189 found in nodules larger than 3mm (41). Taken together, these results suggest that certain
190 APA, particularly those harbouring *CACNA1D*, *ATP1A1* and *ATP2B3* mutations, could derive
191 from APCC, although the precise mechanism remains to be identified. The progression of
192 APCC to APA could be the result of a single-somatic mutation event or alternatively require a
193 second-hit mutation within the APCC to promote cell proliferation. However, it has to be
194 taken into consideration that not all mutations identified in APCCs or pAATL affect amino
195 acids recurrently mutated in APA; those mutations therefore require functional validation for
196 their implication in promoting aldosterone biosynthesis and eventually cell proliferation.

197

198 **The new physiology of aldosterone production - autonomous aldosteronism with age?**

199 In human adrenals, not all cells express *CYP11B2*, possibly reflecting high salt intake in the
200 general population (34). Two patterns of *CYP11B2* expression have been described in the
201 “normal” adrenal gland: a discontinuous expression in scattered cells underneath the capsule
202 and expression in cell clusters named APCC (28, 29). They are composed of subcapsular zona
203 glomerulosa-like cells and inner large zona fasciculata-like cells, expressing *CYP11B2* but not
204 *CYP11B1* (28, 29). Despite the inner portion resembling to zona fasciculata, their
205 transcriptome very much resembles to the transcriptome of zona glomerulosa cells, but with
206 an enhanced capacity to produce aldosterone (39, 41). It has been postulated that APCC are
207 structures of constitutive aldosterone biosynthesis, independent of renin and angiotensin II
208 stimulation (39). This is supported by the fact that APCC in normal adrenal glands harbour
209 somatic mutations in a subset of APA driver genes (39) and that APCC are present in the
210 normal adrenal as well as in peritumoral tissue of adrenals with APA (28, 29). Of note,
211 increased expression of the melanocortin 2 receptor (MC2R) has been reported in APCC,
212 suggesting that adrenocorticotrophic hormone (ACTH) could play a role in the regulation of
213 aldosterone biosynthesis in APCC (39).

214 To assess the evolution of the adrenal cortex structure and function with time, Nishimoto et al
215 have investigated the presence of APCC in adrenal tissues from 33 autopsied patients aged
216 between 0 and 50 years (43). Adrenals from subjects under 11 years had layered zona
217 glomerulosa and zona fasciculata without apparent APCC. The number and size of APCC
218 increased with age. These results suggest either that a somatic mutation occurring in the zona
219 glomerulosa blocks transdifferentiation to zona fasciculata and promotes APCC formation, or
220 that APCC may develop due to aging and environmental factors, with somatic mutations
221 being induced by unknown factors.

222 Based on the assumption that APCC are structures of constitutive aldosterone biosynthesis,
223 the relation of autonomous aldosterone production, APCC and age has been investigated by

224 evaluating the relationship between age and adrenal aldosterone synthase expression in 127
225 adrenal glands from deceased kidney donors aged 9 months to 68 years (44). This study
226 confirmed that continuous expression of CYP11B2 was more frequent in young adrenal
227 glands, while old adrenals very often showed discontinuous or absent zona glomerulosa
228 CYP11B2 expression. Total and normal zona glomerulosa CYP11B2-expressing areas in the
229 adrenal cortex were negatively correlated with age, while the area of CYP11B2 expression in
230 APCC increased. The effect of age on aldosterone production was in parallel investigated in
231 the HyperPATH study population, which consists of normotensive or mildly hypertensive
232 subjects who underwent a high salt and low salt diet (45). On a high salt diet, subjects without
233 PA showed a progressive decline of renin activity with age, while serum and 24-hour urinary
234 aldosterone levels did not change; as a consequence, the aldosterone-to renin ratio was
235 positively associated with older age. Under sodium restriction, the physiological stimulation
236 of both plasma renin activity and aldosterone was blunted with increasing age. Altogether,
237 these findings suggest a progressive pattern of abnormal aldosterone physiology with aging.
238 The presence of APCC, which are supposedly renin-independent aldosterone producing
239 structures, manifests with renin-independent aldosterone secretion and a decreased capacity of
240 the adrenal gland to respond to physiological stimuli with age. The authors suggest that aging
241 may be associated with a subclinical form of aldosterone excess. The fact that total
242 aldosterone-expressing area in the adrenal cortex rather diminishes and aldosterone levels do
243 not significantly change with age in the HyperPATH population rather suggests an
244 inappropriate aldosterone production to salt status or an appropriate relatively autonomous
245 aldosterone production.

246 To gain insight into how APCC evolve with age in subjects without hypertension, Omata et al
247 investigated 107 autaptic adrenal glands using CYP11B2 immunohistochemistry and NGS
248 and determined the APCC score and the associated somatic mutations in non-hypertensive

249 patients from Japanese origin (46). The number of APCCs, which was in average 0.6 per
250 gland, increased with age, without difference in sex distribution. 21 of 61 APCCs (34%)
251 showed somatic mutations in one of the APA driver genes except *KCNJ5*, with 16 affecting
252 *CACNA1D* (76%), which therefore appears to be the most frequently mutated gene in APCCs
253 in healthy individuals from different origins (39) as well as micronodules from image-
254 negative adrenals removed on the basis of positive lateralization of aldosterone production at
255 AVS (40). As only one third of the genetic variants identified have previously been described
256 in APA and functionally characterized, the consequences of the remaining somatic mutations
257 detected in APCC warrants further examination. Remarkably, in four adrenal glands different
258 somatic mutations were identified in individual APCCs, supporting a different clonal origin of
259 each structure, similar to what observed in multiple nodules in multinodular adrenals with
260 APA (32). The results reported here suggest that APCC accumulation may be an age-
261 dependent phenomenon in non-hypertensive subjects, possibly explaining some cases of
262 inappropriate aldosterone secretion not associated with hypertension. As their number was
263 lower in normotensive compared to CT-negative PA patients (40), the authors suggest that
264 APCC may possibly contribute to hypertension not related to PA. Whether APCC may
265 explain some cases of low-renin hypertension requires further investigation.

266

267 **Conclusion**

268 After a large number of studies identifying inherited and somatic mutations in familial
269 hyperaldosteronism and APA, the last years have been devoted to the understanding of the
270 functional consequences of these mutations, their possible clinical relevance and screening, and
271 the search of pharmacological approaches specifically targeting mutated proteins. Exciting new
272 knowledge has also been gathered on normal adrenal cortex structure and function in relation
273 to aldosterone production and the genetics of APCC. It can be anticipated that this knowledge
274 will be applied to patients with inherited or sporadic PA in the next few years. In addition, new
275 genes should be identified in patients with as yet unknown genetic defects, which represent half
276 of the patients harboring an APA and a large proportion of patients with familial
277 hyperaldosteronism. The causes underlying the development of bilateral adrenal hyperplasia
278 and idiopathic hyperaldosteronism remain still to be identified.

279

280 **Key points**

281

- 282 • Four Mendelian forms of hyperaldosteronism, inherited as autosomal dominant traits,
283 have been described
- 284 • Inherited mutations of different genes coding for ion channels and ATPases have been
285 identified in familial hyperaldosteronism; similar somatic mutations are found in APA
- 286 • Pre-operative detection of somatic mutations may allow improved diagnosis and
287 treatment of PA due to APA
- 288 • APCC are autonomous aldosterone-producing structures of the normal adrenal
289 harboring somatic mutations in APA driver genes
- 290 • The aging adrenal may progressively show features of autonomous aldosterone
291 production

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293

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303

304

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306

307

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476 number and size of APCC with aging in non-hypertensive Japanese subjects.

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479 **Figure legends.**

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481 **Figure 1. Two models for APA development.** Foci are composed of zona glomerulosa cells
482 where aldosterone production is under the control of its secretagogues, principally angiotensin
483 II (AngII) and potassium (K⁺). On the left part of the adrenal, the “two-hit model”. Local
484 abnormal cell proliferation occurs in some zona glomerulosa cells, due to activation of specific
485 signaling pathway such as Wnt/ β -catenin or shh, leading to nodule formation. Subsequently,
486 recurrent somatic mutations in *KCNJ5*, *CACNA1D*, *ATP1A1* or *ATP2B3* occur, inducing
487 autonomous aldosterone hypersecretion and APA development. On the right part of the adrenal,
488 the “APCC model”. Somatic mutations in *CACNA1D* or *ATP1A1* lead to constitutive
489 aldosterone production in specific structures named APCC. APCC develop into pAATL and
490 then into APA, through abnormal cell proliferation driven by the previously described somatic
491 mutations or by other mechanisms, which remain to be identified.

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