



**HAL**  
open science

## Genetic and Genomic Mechanisms of Primary Aldosteronism

Fabio L Fernandes-Rosa, Sheerazed Boulkroun, Maria-Christina Zennaro

► **To cite this version:**

Fabio L Fernandes-Rosa, Sheerazed Boulkroun, Maria-Christina Zennaro. Genetic and Genomic Mechanisms of Primary Aldosteronism. *Trends in Molecular Medicine*, 2020, 26 (9), pp.819-832. 10.1016/j.molmed.2020.05.005 . inserm-03554636

**HAL Id: inserm-03554636**

**<https://inserm.hal.science/inserm-03554636>**

Submitted on 3 Feb 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

## **Genetic and genomic mechanisms of primary aldosteronism**

Fabio L. Fernandes-Rosa<sup>1</sup>, Sheerazed Boulkroun<sup>1</sup>, Mariani-Christina Zennaro<sup>1,2</sup>

### **Affiliations:**

<sup>1</sup> Inserm, PARCC, Université de Paris, F-75015 Paris, France

<sup>2</sup>Assistance Publique-Hôpitaux de Paris, Hôpital Européen Georges Pompidou, Service de Génétique, Paris, France

Address correspondence to:

Maria-Christina Zennaro, MD, PhD

INSERM, U970

Paris Cardiovascular Research Center – PARCC

56, rue Leblanc,

75015 Paris – France

Tel : +33 (0)1 53 98 80 42

Fax : + 33 (0)1 53 98 79 52

e-mail: [maria-christina.zennaro@inserm.fr](mailto:maria-christina.zennaro@inserm.fr)

### **Disclosures**

The authors have nothing to disclose.

## **Abstract**

Aldosterone producing adenomas (APA) and bilateral adrenal hyperplasia are the main cause of primary aldosteronism (PA), the most frequent form of secondary hypertension. Mutations in ion channels and ATPases have been identified in APA and inherited forms of PA, highlighting the central role of calcium signalling in PA development. Different somatic mutations are also found in aldosterone producing cell clusters in adrenals from normal subjects and from patients with unilateral and bilateral PA, suggesting additional pathogenic mechanisms. Recent mouse models have also contributed to a better understanding of PA. Application of genetic screening in familial PA, development of surrogate biomarkers for somatic mutations in APA and use of targeted treatment directed at mutated proteins may allow improved management of patients.

1 Primary aldosteronism (PA) is the most frequent form of secondary arterial hypertension  
2 with a prevalence of 5% of hypertensive patients in primary care and until 10% of hypertensive  
3 subjects in reference centers [1, 2]. PA is due to autonomous aldosterone production by the  
4 adrenal gland, the two major causes being the presence of an aldosterone producing adenoma  
5 (APA) or bilateral adrenal hyperplasia (BAH). In addition to sporadic forms, four different  
6 familial forms were described, accounting for approximately 5% of PA cases. PA patients  
7 exhibit hypertension associated in many cases with hypokalemia. The biochemical diagnosis is  
8 based on high levels of plasma aldosterone and low plasma renin, with an increased aldosterone  
9 to renin ratio (ARR). The confirmation of PA diagnosis is performed by one of different tests,  
10 including oral salt loading or captopril challenge tests, followed by adrenal image and adrenal  
11 vein sampling (AVS) to subtype PA as unilateral or bilateral forms [3]. PA subtyping guides  
12 the optimal treatment, unilateral adrenalectomy for lateralized APA or long-life drug therapy  
13 with mineralocorticoid receptor antagonists in the presence of bilateral forms. Due to  
14 deleterious effects of aldosterone on blood vessels and heart, patients with PA have an increased  
15 cardiovascular risk when compared with age, sex and blood pressure matched patients with  
16 primary hypertension [4, 5].

17 Aldosterone is synthesized from cholesterol in the cells of the zona glomerulosa (ZG) of  
18 the adrenal cortex by a cascade of enzymatic reactions. The ZG specifically expresses the  
19 enzyme aldosterone synthase (encoded by *CYP11B2*), responsible for the three final enzymatic  
20 reactions leading to aldosterone biosynthesis [6]. Aldosterone biosynthesis is tightly regulated  
21 to maintain salt and water homeostasis, the two most important factors being angiotensin II  
22 (AngII) and extracellular potassium concentration ( $K^+$ ) (Box 1). In basal conditions ZG cells  
23 are maintained in a hyperpolarized state due to the presence of a large number of potassium  
24 channels [7] and the  $Na^+/K^+$ -ATPase. ZG cell membrane depolarization is induced by AngII or  
25 increase in plasma  $K^+$  concentration, causing the opening of voltage-gated  $Ca^{2+}$  channels and  
26 the increase of the intracellular  $Ca^{2+}$  concentration. AngII also stimulates  $Ca^{2+}$  release from the  
27 endoplasmic reticulum by binding the angiotensin II Type 1 receptor (AT1R) and stimulating  
28 the inositol triphosphate pathway. The increase in cytosolic  $Ca^{2+}$  activates calcium signaling  
29 (via calmodulin and calcium/calmodulin-dependent protein kinases), the main trigger for  
30 *CYP11B2* expression and aldosterone production [8, 9]. Other regulators of aldosterone  
31 biosynthesis include ACTH, serotonin, interleukine 6, epidermal growth factor and leptin [10-  
32 13].

33 In the last decade, following the discovery of *KCNJ5* mutations associated with PA,  
34 different studies performing whole exome sequencing identified mutations in genes coding for  
35 ion channels and ATPases as responsible for APA and familial PA and highlighted the central  
36 role of calcium signaling on PA pathogenesis. These studies allowed the identification of  
37 somatic mutations in more than 85% of APA and completely changed the classification of  
38 familial forms (Box 2. Clinician's Corner).

39

#### 40 **Genetics of primary aldosteronism**

41 The first genetic defect associated with PA was described almost 30 years ago. A chimeric  
42 gene, where the regulatory regions of *CYP11B1* are juxtaposed with the coding sequence of  
43 *CYP11B2*, was identified as responsible of familial hyperaldosteronism Type 1 (FH-I) [14, 15]  
44 (Table 1). FH-I is an autosomal dominant disease presenting as early-onset hypertension  
45 associated with biochemical abnormalities of PA and increased production of 18-  
46 hydroxycortisol and 18-oxocortisol, which improves with glucocorticoid treatment [16, 17].  
47 The chimeric *CYP11B1/CYP11B2* gene leads to ectopic expression of *CYP11B2* throughout the  
48 adrenal cortex, with aldosterone being produced not only in cells from ZG, and with an  
49 inappropriate ACTH-dependent regulation of aldosterone biosynthesis following the circadian  
50 rhythm of cortisol (Figure 1) [18, 19].

51 During the last decade, whole exome sequencing (WES) performed on DNA from APA  
52 has allowed the identification of somatic mutations in genes coding for ion channels (*KCNJ5*  
53 encoding for the potassium channel GIRK4, *CACNA1D* coding for the  $\alpha 1$  subunit of the L-type  
54  $\text{Ca}^{2+}$  channel Cav1.3) and ATPases (*ATP1A1*, coding for the  $\alpha 1$  subunit of the  $\text{Na}^+, \text{K}^+$ -ATPase,  
55 and *ATP2B3*, coding for the plasma membrane calcium-transporting ATPase 3 (PMCA3) [20-  
56 23]. The frequency of somatic mutations was reported to be approximately 50% in studies  
57 performing Sanger sequencing of hot spot regions of APA-driver genes on DNA extracted from  
58 frozen pieces of tumor [24, 25]. The recent development of aldosterone synthase (*CYP11B2*)  
59 immunohistochemistry (IHC)-guided next-generation sequencing (NGS) allowed the  
60 identification of mutations in APA-driver genes in 88% to 93% of APA [26-28].

61 In parallel, the discovery of germline mutations in genes coding for the same ion channels  
62 identified in APA and also in *CACNA1H* (coding for the  $\alpha 1$  subunit of the T-type  $\text{Ca}^{2+}$  channel  
63 Cav3.2) and *CLCN2* (coding for the chloride channel ClC-2) have complexified the

64 classification of familial forms of PA [20, 22, 29-32], with four different forms now classified  
65 in FH-I to FH-IV, based on the underlying genetic defect.

66 All the different somatic and germline mutations increase *CYP11B2* expression and  
67 aldosterone biosynthesis by affecting cell membrane potential and/or intracellular ionic  
68 homeostasis, resulting in increased intracellular calcium concentration and activation of  
69 calcium signaling (Figure 1).

#### 70 ***KCNJ5 mutations***

71 Somatic and germline *KCNJ5* mutations were identified in APA and in patients with a  
72 familial form of PA (FH-III) [20]. The most prevalent *KCNJ5* mutations (p. Gly151Arg, p.  
73 Thr158Ala, p. Leu168Arg) are localized near or within the selectivity filter of the channel.  
74 These mutations cause loss of K<sup>+</sup> selectivity and increase sodium permeability, resulting in cell  
75 membrane depolarization followed by opening of voltage-gated Ca<sup>2+</sup> channels and stimulation  
76 of Ca<sup>2+</sup> signaling [20, 33, 34] (Figure 1). A small number of *KCNJ5* mutations are localized in  
77 regions far from the selectivity filter and could be responsible for decreased abundance of the  
78 channel at the plasma membrane [35]. While the impact of GIRK4 downregulation on  
79 aldosterone production was not observed in cell models [33], a recent study suggested that cell  
80 proliferation and apoptosis may be regulated by the level of GIRK4 expression [36].

81 Recurrent somatic *KCNJ5* mutations are the most frequent genetic abnormality observed  
82 in APA, being identified in approximately 40% of adenomas [24, 37, 38]. Ethnic differences  
83 were observed among cohorts, with a higher prevalence of somatic *KCNJ5* mutations in patients  
84 from East Asian cohorts [35, 39-41]. Different studies have shown that *KCNJ5* mutations are  
85 more frequent in female and younger patients [24-28, 35, 38, 42, 43], and may also be associated  
86 with higher aldosterone levels and larger tumor size in some studies [37, 38] but not in others  
87 [28]. *KCNJ5* mutations were also suggested to be a marker of better surgical outcome after  
88 adrenalectomy [44]. Compared with other mutational groups, APA carrying *KCNJ5* mutations  
89 present lower percentage of cells expressing CYP11B2 and higher percentage of cells  
90 expressing CYP11B1 [28]. A recent study, however, reported no difference in overall  
91 aldosterone synthase expression as a function of mutational status [45]. In addition, *KCNJ5*-  
92 mutated APA exhibit decreased GIRK4 expression in comparison with the adjacent ZG [28,  
93 36, 46, 47].

94 Germline *KCNJ5* mutations are also responsible for FH-III [20] (Table 1). FH-III was first  
95 described in 2008 as severe early-onset hypertension associated with hypokalemia, high urinary  
96 concentrations of hybrid steroids 18-oxocortisol and 18-hydroxycortisol, and massive bilateral

97 adrenal hyperplasia [48]. Germline *KCNJ5* mutations were found in different FH-III families  
98 presenting variability in the severity of hyperaldosteronism and in the subtype of PA, sometimes  
99 within the same family [49-53].

100 *In vitro* studies revealed that mutated GIRK4 may be blocked by high therapeutic doses of  
101 the calcium channel blocker verapamil, or by macrolides, decreasing *CYP11B2* expression and  
102 aldosterone biosynthesis [54-56]. Clinical studies are currently ongoing, in hypertensive  
103 patients with PA, to evaluate the potential clinical use of macrolides on PA subtyping or the  
104 identification of somatic *KCNJ5* mutations before surgery [57].

### 105 *CACNAID and CACNAIH mutations*

106 *CACNAID* mutations affect different properties of Cav1.3 channel, delaying the voltage-  
107 dependent inactivation or inducing opening of the channel at less depolarized potentials. This  
108 results in increased Ca<sup>2+</sup> influx leading to the activation of Ca<sup>2+</sup> signaling and aldosterone  
109 overproduction (Figure 1) [21, 22]. Using CYP11B2 IHC-guided NGS, the prevalence of  
110 somatic *CACNAID* mutations was 21% in APA from white American patients, 37% in a French  
111 cohort, and as high as 42% in APA from African-American patients, being the most frequent  
112 genetic abnormality in this population [26-28]. While *CACNAID* mutations were associated  
113 with smaller tumors in studies using Sanger sequencing to genotype APA [24], this association  
114 was not described in a recent study using CYP11B2 IHC-guided NGS [28]. In addition to  
115 somatic mutations, *de novo* germline mutations in *CACNAID* were identified in two children  
116 exhibiting a severe form of hyperaldosteronism in the first year of life associated with a  
117 complex neurologic disorder (Primary Aldosteronism, Seizures and Neurologic Abnormalities  
118 (PASNA) [22] (Table 1).

119 Germline *CACNAIH* mutations are responsible for FH-IV. Mutations modify the Cav3.2  
120 channel properties inducing a gain of function, resulting in increased Ca<sup>2+</sup> influx followed by  
121 activation of calcium signaling and overproduction of aldosterone (Figure 1) [29, 30]. The  
122 recurrent germline *CACNAIH* mutation p.Met1549Val was described in five children with  
123 onset of PA before age 10 years [29]. Micronodular adrenal hyperplasia was observed in the  
124 histological analysis of one case [29]. A *de novo* germline *CACNAIH* mutation also affecting  
125 codon 1549 (p.Met1549Ile) was described in one children with PA associated with multiplex  
126 developmental disorder [30]. Germline *CACNAIH* mutations were also identified in adult  
127 patients with a familial inheritance of PA and in one patient with APA, suggesting that  
128 *CACNAIH* mutations may predispose to PA with different phenotypic presentation [30].  
129 Recently, somatic *CACNAIH* mutations were also observed in a rare subset of APA [58].

130

### 131 *ATP1A1 and ATP2B3 mutations*

132 *ATP1A1* mutations were identified in 8% to 17% of APA by CYP11B2 IHC-guided NGS  
133 [26-28]. The  $\alpha$  subunit of the  $\text{Na}^+, \text{K}^+$ -ATPase, encoded by *ATP1A1*, is composed of 10  
134 transmembrane domains (M1 to M10) and intracellular N- and C- termini. Somatic *ATP1A1*  
135 mutations are localized in M1, M4 and M9 domains [21, 23, 25, 40, 59]. While mutations in  
136 the M1 and M4 domains affect the  $\text{K}^+$  binding pocket decreasing the pump affinity for  $\text{K}^+$ ,  
137 mutations in the M9 domain affect a supposed sodium-specific site of the pump, all mutations  
138 decreasing the pump activity [60, 61]. The reduced affinity for  $\text{K}^+$  and the loss of pump activity  
139 result in cell membrane depolarization and cell acidification, without overt increase of  
140 intracellular  $\text{Ca}^{2+}$  (Figure 1) [60]. However, the role of cytosolic acidification on autonomous  
141 aldosterone production is not established.

142 *ATP2B3* mutations were identified in 1.7% to 10% of APA, with higher prevalence in  
143 studies using CYP11B2 IHC-guided NGS [24-28, 43]. PMCA3, encoded by *ATP2B3* is  
144 composed of 10 transmembrane domains (M1 to M10) and intracellular N- and C- termini. All  
145 APA-driver *ATP2B3* mutations are in frame deletions affecting a “PEGL” motif in the M4  
146 domain, a region involved in calcium binding and ion gating [23-25, 43, 62]. Mutations in  
147 *ATP2B3* decrease pump activity, impairing calcium export and resulting in increased cytosolic  
148 calcium concentration and activation of calcium signaling (Figure 1). A second mechanism for  
149 autonomous aldosterone biosynthesis was suggested; *ATP2B3* mutations may induce a sodium  
150 leak resulting in cell membrane depolarization and opening of voltage-dependent calcium  
151 channels, but may also induce a calcium leak through mutated pumps [63].

### 152 *CLCN2 mutations*

153 Germline *CLCN2* mutations are responsible for FH-II (Table 1). FH-II was used to group  
154 all familial forms of PA without an established genetic cause, with a diagnosis based on the  
155 presence of two or more affected family members [64, 65]. Whole-exome sequencing  
156 performed in a large Australian FH-II family identified a germline mutation in *CLCN2*  
157 segregating with the disease in 8 subjects [32]. Three other PA pedigrees carried the same  
158 mutation and, additionally, four different germline *CLCN2* mutations were identified in  
159 unrelated patients with early-onset PA [32]. A *de novo* germline *CLCN2* mutation was  
160 identified, concomitantly, in a 9 years-old PA patient exhibiting severe hypertension and  
161 profound hypokalemia [31]. This mutation changes the properties of ClC-2 leading to increased  
162 chloride conductance at resting potentials and chloride efflux followed by cell membrane

163 depolarization, activation of calcium signaling and stimulation of *CYP11B2* expression and  
164 autonomous aldosterone production (Figure 1) [31]. In addition to germline mutations and FH-  
165 II, a somatic *CLCN2* mutation was reported in one APA [66].

166

### 167 ***CTNNB1, PRKACA and ARMC5 mutations***

168 Somatic mutations in *CTNNB1*, coding for  $\beta$ -catenin, were identified in 2.1% to 5.1% of  
169 APA [43, 67]. *CTNNB1* mutations are localized in exon 3 and were previously described in  
170 cortisol-producing adenomas and adrenocortical cancer. *CTNNB1* mutations are more prevalent  
171 in women and an association with PA during pregnancy due to aberrant expression of gonadal  
172 receptors in APA was suggested [68]. However, it has been shown that the regulation of  
173 aldosterone production by gonadal hormones may also occur in APA without somatic *CTNNB1*  
174 mutations [69]. Further studies are necessary to establish the role of *CTNNB1* mutations on  
175 APA development.

176 Somatic *PRKACA* (encoding the cAMP-dependent protein kinase catalytic subunit alpha)  
177 mutations were identified in two patients with APA, one presenting aldosterone and cortisol co-  
178 secretion [70]. *PRKACA* mutations were also described in cortisol producing adenomas and  
179 their roles in autonomous aldosterone production and APA development are unknown.

180 Finally, germline *ARMC5* (armadillo repeat containing 5) variants, predicted to be  
181 damaging, were observed in African-American patients with PA [71]. Germline *ARMC5*  
182 variants were also observed in a European cohort of patients with PA, although they were not  
183 predicted to be damaging using bioinformatics tools [72]. *ARMC5* mutations were previously  
184 identified in primary bilateral macronodular adrenal hyperplasia and associated with  
185 hypercortisolism [73]. The link between *ARMC5* mutations and PA remains to be further  
186 determined.

### 187 **Animal models of primary aldosteronism**

188 Genetic studies have greatly contributed to a better understanding of the mechanisms  
189 responsible for PA development, highlighting the role of ion channels and pumps, but also of  
190 the Wnt/ $\beta$ -catenin pathway (Table 2). Remarkably, potassium channels and the Wnt/ $\beta$ -catenin  
191 pathway had been shown previously to play crucial roles in the regulation of aldosterone  
192 biosynthesis and homeostasis of the adrenal cortex by specific mouse models.

193 Invalidation of *kcnk3* or/and *kcnk9*, coding for the two-pore domain potassium channels  
194 Task1 and Task3, resulted in different forms of hyperaldosteronism or low renin hypertension

195 [74-78]. Simultaneous invalidation of *kcnk3* and *kcnk9* leads to ZG cell depolarization and PA  
196 resistant to salt suppression, without affecting cellular morphology and adrenal zonation [75].  
197 ZG-specific invalidation of both potassium channels resulted in a milder phenotype consisting  
198 of a moderate increase of plasma aldosterone that was not associated with a reduction of plasma  
199 renin or increased ARR; nevertheless, it was sufficient to significantly increase blood pressure  
200 [78]. Different phenotypes were observed when *Kcnk3* or *kcnk9* were invalidated separately.  
201 Task3 channel deletion causes salt-sensitive hypertension with low renin [76, 77], associated  
202 with ZG cell depolarization, modification of Ca<sup>2+</sup> signaling and alteration of the physiological  
203 regulation of aldosterone, without changes in adrenal morphology. In neonatal mice, Task3  
204 deletion led to major adrenal dysfunction, associated with increased aldosterone, corticosterone  
205 and progesterone levels [79]. Deletion of Task1 resulted in severe hyperaldosteronism  
206 remediable by glucocorticoids associated with abnormal expression of aldosterone synthase in  
207 the zona fasciculata (ZF), despite normal morphological zonation of the adrenal cortex [74].  
208 Whereas this phenotype was observed in female mice all along their life, it was only observed  
209 in pre-pubertal male mice, suggesting an androgen-driven upregulation of compensatory  
210 mechanisms [74]. In these mice, a subset of genes was closely associated with  
211 hyperaldosteronism, including *Dkk3* coding for Dickkopf3, a member of the Wnt signaling  
212 pathway [80]. Interestingly, invalidation of *dkk3* in *kcnk3*<sup>-/-</sup> mice resulted in hyperaldosteronism  
213 also in male mice, suggesting a role for *dkk3* in the development of PA [80].

214 After the identification of *CLCN2* mutations in patients with FH-II, two different knock-in  
215 mouse models provided important insight regarding the role of chloride channels in PA  
216 development [81, 82]. Deletion of eight N-terminal residues constituting the inactivation  
217 domain of the Clc-2 chloride channel, where the p.Met22Lys, p.Gly24Asp and p.Tyr26Asn  
218 mutations are located [31, 32], resulted in hypertension and hypokalemia in mice, with high  
219 aldosterone and low renin levels, recapitulating the major features of PA [81]. Mice harboring  
220 the heterozygous p.Arg180Gln mutation, mimicking the human p.Arg172Gln mutation found  
221 in FH-II [32], present a mild form of PA with increased aldosterone associated with a mild  
222 elevation of blood pressure, but absence of morphological adrenal abnormalities [82].

223 Through different mechanisms, the mutations identified in APA converge upon increased  
224 calcium signaling to increase aldosterone production. The constitutive expression in mice of a  
225 gain-of-function mutant of the AII receptor type 1A (AT1R) led to the development of low-  
226 renin hypertension and relative hyperaldosteronism [83]. More recently, specific activation of  
227 Gq signaling in adrenal cortex, using a Designer Receptors Exclusively Activated by Designers

228 Drugs (DREADD) activated by clozapine N-oxide (CNO), was used to establish a mouse model  
229 of PA [84]. CNO treatment results in increase of Cyp11b2 expression and aldosterone levels,  
230 decreased plasma renin concentration and abnormal zonation of Cyp11b2, with ZF cells  
231 expressing both Cyp11b1 and Cyp11b2. In addition, high blood pressure was observed after  
232 CNO treatment in mice submitted to high salt diet.

233 Whereas these mouse models develop hyperaldosteronism, the presence of tumors was  
234 never observed, suggesting that modification of intracellular ion balance is not sufficient to  
235 induce both increase of aldosterone production and adenoma development. Activation of the  
236 Wnt/ $\beta$ -catenin pathway, playing an important role in adrenal development, has been involved  
237 in adrenal carcinoma and adenoma [85] and was found in two-thirds of APA [86]. In mice,  
238 constitutive activation of  $\beta$ -catenin results in adrenal hyperplasia and dysplasia as well as  
239 increased cell proliferation. More interestingly,  $\beta$ -catenin activation also led to increased  
240 aldosterone production and hyperaldosteronism [87]. However, the development of tumors was  
241 observed only in rare cases with features of carcinoma rather than adenoma [87].

242 Recently, retinoic acid receptor  $\alpha$  (RAR $\alpha$ ) signaling has been identified as involved in  
243 nodule formation by transcriptome analysis performed in APA. Some years ago, proteomic  
244 analyses have suggested a role of retinoic acid receptor signaling in the early stage of adrenal  
245 development [88]. Invalidation of rar $\alpha$  expression in mice results in structural disorganization  
246 of the adrenal cortex characterized by the loss of the radial organization of the ZF and  
247 abnormalities in vessel architecture and extracellular matrix, in both male and female mice,  
248 with no major modifications of aldosterone production [89]. Beyond the role of rar $\alpha$  in the  
249 maintenance of the normal adrenal cortex structure and cell proliferation, dysregulation of this  
250 signaling may contribute to abnormal cell proliferation in PA [89].

251

### 252 ***APCC and their role in aldosterone biosynthesis and PA***

253 Until the description of aldosterone producing cell clusters (APCC), expression of  
254 *CYP11B2* and aldosterone biosynthesis was thought to occur exclusively in ZG cells under the  
255 tight control of different factors, the two majors being AngII and K<sup>+</sup> (see above). APCC have  
256 been described in 2010 [90]. They are located under the capsule and composed of ZG-like cells  
257 in the outer part and of columnar ZF-like cells in the inner part. Despite their morphological  
258 properties and the fact that Disabled-2, a marker of ZG, is expressed only in the outer part, the  
259 entire structure expresses CYP11B2 but not CYP11B1, suggesting that APCC may constitute

260 intermediate structure between ZG and ZF [86, 91]. However, transcriptome profiling of APCC  
261 revealed a greater homology to ZG cells than to ZF cells [92, 93]. APCC are found in normal  
262 adrenals and their number and size are correlated with age, but not with gender [45]. APCC are  
263 also found in adrenal cortex adjacent to an APA and in adrenals from patients with BAH [90,  
264 91, 94].

265 Genetic analysis of APCC, using CYP11B2 IHC-guided guided NGS on FFPE tissue,  
266 identified somatic mutations in APA driver genes, including *CACNA1D*, *ATP1A1* and *ATP2B3*,  
267 but not *KCNJ5*, in 35% of APCC from normal adrenals [92]. More recently, the description of  
268 APCC-to-APA translational lesions (pAATL), composed of a subcapsular APCC-like structure  
269 and a micro-APA-like structure and carrying somatic mutations suggested the possibility for  
270 APCC to constitute the starting point for the development of APA (Figure 2) [93]. Other  
271 evidences, however, support the occurrence of two independent events leading to APA  
272 development [95]. It has been shown that the adrenal cortex adjacent to an APA may exhibit  
273 ZG hyperplasia, increased nodulation and reduced vascularization [86]. Distinct CYP11B2  
274 positive nodules may carry different mutations within the same adrenal [28, 96] and different  
275 APCC located in the cortex adjacent to an APA may also carry different somatic mutations,  
276 including *KCNJ5* mutations [28]. Finally, a patient with lateralized PA and familial  
277 adenomatous polyposis further supports a two-hit model for APA development. The patients  
278 carried a germline *APC* mutation with loss of heterozygosity in adrenals (first hit), responsible  
279 for a macronodular hyperplasia. In one CYP11B2 positive nodule, a somatic *KCNJ5* mutation  
280 was identified, being responsible for autonomous aldosterone production (second hit) (Figure  
281 2) [97].

282 APCC appear also to play a role in the pathogenesis of bilateral forms of PA. In a study  
283 investigating adrenals from 15 patients with BAH who underwent adrenalectomy, the number  
284 of APCC was significantly higher compared to adrenals from normotensive controls,  
285 suggesting that APCC, through autonomous aldosterone production, may contribute to the  
286 development of PA. Interestingly, sequencing of 99 APCC from the 15 adrenals identified  
287 somatic mutations in at least one APCC in all but one case; all mutations affected the *CACNA1D*  
288 gene [94]. These results suggest for the first time that the presence of APCC in BAH may be,  
289 in part, responsible for PA development; however, they need to be confirmed in a larger cohort  
290 of patients.

291

292 **Concluding remarks**

293

294 Over the last years, CYP11B2 guided NGS has allowed the identification of somatic  
295 mutations in APA-driver genes in more than 85% of APA. In addition, the description of  
296 germline mutations in genes coding for different ion channels has allowed refining the  
297 classification of familial forms of PA and the description of *de novo* early-onset PA. In  
298 mechanistic terms, these mutations highlight the central role of plasma membrane potential,  
299 intracellular calcium concentrations and activation of calcium signaling in the pathogenesis of  
300 PA. However, the sequence of events leading to APA formation as well as the mechanisms  
301 responsible for bilateral PA remains unknown. Somatic mutations in APA-driver genes were  
302 also identified in APCC from normal adrenals and from adrenals with APA. While this  
303 discovery suggests a possible role of APCC in PA, the identification of different mutations  
304 among APA, APCC and micronodules within the same adrenal implies the contribution of  
305 additional mechanisms (see Outstanding Questions). Finally, the investigation of different  
306 mouse models has largely contributed to our understanding of some aspects of the development  
307 of hyperaldosteronism. However, none of these models leads to APA development, suggesting  
308 that there might be the requirement for additional events to trigger nodulation, which remain to  
309 be established. New studies identifying surrogate markers of APA mutational status before  
310 surgery, associated with the recent description of drugs specifically blocking mutated KCNJ5  
311 channels, could open new and exciting perspectives for a better diagnosis and targeted treatment  
312 of PA.

313

#### 314 **Acknowledgments**

315 The laboratory of Dr. Maria-Christina Zennaro is supported through institutional funding from  
316 INSERM, by the Agence Nationale pour la Recherche (ANR-15-CE14-0017-03, ANR-18-  
317 CE93-0003-01), the Fondation pour la Recherche Médicale (EQU201903007864) and by the  
318 H2020 project ENSAT-HT grant n° 633983.

319

320

321 **Box 1. Regulation of aldosterone biosynthesis**

322 Aldosterone is produced in the zona glomerulosa of the adrenal cortex to maintain blood volume  
323 as well as sodium and potassium homeostasis in the body. Aldosterone is synthesized from  
324 cholesterol through sequential enzymatic steps catalyzed by different cytochrome P450  
325 enzymes and hydroxysteroid dehydrogenases. In the zona glomerulosa, the enzyme aldosterone  
326 synthase (encoded by *CYP11B2*) catalyzes the three terminal steps of aldosterone biosynthesis:  
327 hydroxylation at position C11 of deoxycorticosterone (DOC) resulting in corticosterone,  
328 hydroxylation at position C18 of corticosterone yielding 18-hydroxy-corticosterone and the  
329 oxidation of the hydroxyl group at C18, producing aldosterone.

330 Aldosterone biosynthesis is mainly regulated by the renin angiotensin system and plasma  
331 potassium concentrations. Under basal condition zona glomerulosa cells are hyperpolarized,  
332 due to the presence of a large number of  $K^+$  channels. Both increased extracellular potassium  
333 concentrations and angiotensin II inhibit  $K^+$  channels, leading to cell membrane depolarization,  
334 opening of voltage gated  $Ca^{2+}$  channels, increasing intracellular  $Ca^{2+}$  concentrations.  
335 Angiotensin II also signals through the Angiotensin II Type 1 receptor via  $G_{\alpha q}$  proteins leading  
336 to activation of phospholipase C- $\beta$ , increase of inositol triphosphate, and mobilization of  
337 intracellular  $Ca^{2+}$  stores from the endoplasmic reticulum, increasing intracellular calcium  
338 levels. This leads to activation of  $Ca^{2+}$  signaling, which increases aldosterone biosynthesis by  
339 activating transcription factors, in particular the nuclear receptor subfamily 4 group A (NR4A)  
340 members 1 and 2 (NURR77 or NGF1B and NURR1), the cyclic AMP-dependent transcription  
341 factor ATF-1 and the cyclic AMP-responsive element-binding protein (CREB), which increase  
342 the expression of *CYP11B2*, coding for aldosterone synthase. Increased  $Ca^{2+}$  signaling also  
343 enhances early and intermediary steps of adrenal steroidogenesis, including release of  
344 cholesterol from cytoplasmic stores, activation of the expression of the steroid acute regulatory  
345 protein (StAR), which promotes the transfer of cholesterol to the inner mitochondrial  
346 membrane, and the synthesis of cofactors for p450 cytochrome enzyme.

347 **Box 2. Clinician's Corner**

348 Primary aldosteronism (PA) is the most frequent form of secondary arterial hypertension  
349 with a prevalence of up to 10% of hypertensive patients. PA patients have a higher  
350 cardiovascular risk compared to patients with essential hypertension. The two main causes of  
351 PA are unilateral aldosterone producing adenomas (APA) and bilateral adrenal hyperplasia. The  
352 diagnosis of PA includes screening, confirmation tests and subtype identification. Treatment is

353 tailored to the underlying cause: adrenalectomy for unilateral disease and drug therapy for  
354 bilateral forms.

355 Familial forms of PA (FH) account for 5% of the cases. They are classified according to  
356 the underlying genetic defect. FH-I is caused by a chimeric gene composed of the promoter  
357 regions of *CYP11B1* and the coding region from *CYP11B2*. FH-II is caused by germline  
358 mutations in the gene *CLCN2*, coding for the chloride channel ClC-2. Germline mutations in  
359 *KCNJ5*, coding for the potassium channel GIRK4 are responsible FH-III. Finally, germline  
360 mutations in *CACNA1H*, coding for the voltage dependent T-type calcium channel Cav 3.2 are  
361 responsible by FH-IV. Germline *CACNAID* mutations were associated with a rare form of  
362 early-onset hyperaldosteronism associated with neurological abnormalities.

363 Somatic mutations in genes coding for ion channels (*KCNJ5*, *CACNAID*) and ATPases  
364 (*ATP1A1* and *ATP2B3*) were identified in the majority of APA. Their frequency may vary in  
365 different populations, with *KCNJ5* being more frequent in cohorts from Asia and *CACNAID*  
366 mutations in cohorts from African ancestry. *KCNJ5* mutations are more frequent in women and  
367 young patients. Mutations in *CTNNT1*, coding for  $\beta$ -catenin, were identified in 5% of APA,  
368 most frequently in women and possibly associated to pregnancy.

369 Somatic mutations are also found in aldosterone producing cell clusters (APCC), adrenal  
370 structures responsible for autonomous aldosterone production, in normal adrenals. Somatic  
371 mutations in APCC are also found in the adrenal cortex adjacent to APA and adrenals from  
372 bilateral adrenal hyperplasia. APCC may play a role in the development of PA, as well as in the  
373 physiology of aldosterone biosynthesis in normal adrenals.

374 The discovery of the genetic basis of PA opens new perspectives for the development of  
375 new and better diagnostic tools and for drug therapy targeting mutated proteins. Also, it allows  
376 for genetic screening and counseling in affected families and improved management of  
377 mutation carriers.

378

379

380

381 **References**

- 382 1. Monticone, S. et al. (2017) Prevalence and Clinical Manifestations of Primary Aldosteronism  
383 Encountered in Primary Care Practice. *J Am Coll Cardiol* 69 (14), 1811-1820.
- 384 2. Hannemann, A. and Wallaschofski, H. (2012) Prevalence of primary aldosteronism in  
385 patient's cohorts and in population-based studies--a review of the current literature. *Horm*  
386 *Metab Res* 44 (3), 157-62.
- 387 3. Funder, J.W. et al. (2016) The Management of Primary Aldosteronism: Case Detection,  
388 Diagnosis, and Treatment: An Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol*  
389 *Metab* 101 (5), 1889-916.
- 390 4. Savard, S. et al. (2013) Cardiovascular complications associated with primary aldosteronism:  
391 a controlled cross-sectional study. *Hypertension* 62 (2), 331-6.
- 392 5. Monticone, S. et al. (2018) Cardiovascular events and target organ damage in primary  
393 aldosteronism compared with essential hypertension: a systematic review and meta-analysis.  
394 *Lancet Diabetes Endocrinol* 6 (1), 41-50.
- 395 6. Connell, J.M. and Davies, E. (2005) The new biology of aldosterone. *J Endocrinol* 186 (1),  
396 1-20.
- 397 7. Bandulik, S. et al. (2010) TASK1 and TASK3 potassium channels: determinants of  
398 aldosterone secretion and adrenocortical zonation. *Horm Metab Res* 42 (6), 450-7.
- 399 8. Bassett, M.H. et al. (2004) The regulation of aldosterone synthase expression. *Mol Cell*  
400 *Endocrinol* 217 (1-2), 67-74.
- 401 9. Szekeres, M. et al. (2009) Mechanisms of angiotensin II-mediated regulation of aldosterone  
402 synthase expression in H295R human adrenocortical and rat adrenal glomerulosa cells. *Mol*  
403 *Cell Endocrinol* 302 (2), 244-53.
- 404 10. Hattangady, N.G. et al. (2012) Acute and chronic regulation of aldosterone production. *Mol*  
405 *Cell Endocrinol* 350 (2), 151-62.
- 406 11. Kim, S.Y. et al. (1998) EGF-stimulated aldosterone secretion is mediated by tyrosine  
407 phosphorylation but not by phospholipase C in cultured porcine adrenal glomerulosa cells. *J*  
408 *Korean Med Sci* 13 (6), 629-37.
- 409 12. Judd, A.M. et al. (2000) Possible function of IL-6 and TNF as intraadrenal factors in the  
410 regulation of adrenal steroid secretion. *Ann N Y Acad Sci* 917, 628-37.
- 411 13. Lefebvre, H. et al. (2019) Paracrine Regulation of Aldosterone Secretion in Physiological  
412 and Pathophysiological Conditions. *Vitam Horm* 109, 303-339.

- 413 14. Lifton, R.P. et al. (1992) A chimaeric 11beta-hydroxylase aldosterone synthase gene causes  
414 glucocorticoid-remediable aldosteronism and human hypertension. *Nature* 355, 262-265.
- 415 15. Pascoe, L. et al. (1992) Glucocorticoid-Suppressible Hyperaldosteronism Results from  
416 Hybrid Genes Created by Unequal Crossovers Between CYP11B1 and CYP11B2. *Proc. Natl.*  
417 *Acad. Sci. - U.S.A.* 89, 8327-8331.
- 418 16. Sutherland, D.J. et al. (1966) Hypertension, increased aldosterone secretion and low plasma  
419 renin activity relieved by dexamethasone. *Can Med Assoc J* 95 (22), 1109-19.
- 420 17. Halperin, F. and Dluhy, R.G. (2011) Glucocorticoid-remediable aldosteronism. *Endocrinol*  
421 *Metab Clin North Am* 40 (2), 333-41, viii.
- 422 18. Pascoe, L. et al. (1995) Glucocorticoid-suppressible hyperaldosteronism and adrenal tumors  
423 occurring in a single French pedigree. *J Clin Invest* 96 (5), 2236-46.
- 424 19. Stowasser, M. and Gordon, R.D. (2000) Primary aldosteronism: learning from the study of  
425 familial varieties. *J Hypertens* 18 (9), 1165-76.
- 426 20. Choi, M. et al. (2011) K<sup>+</sup> channel mutations in adrenal aldosterone-producing adenomas  
427 and hereditary hypertension. *Science* 331 (6018), 768-72.
- 428 21. Azizan, E.A. et al. (2013) Somatic mutations in ATP1A1 and CACNA1D underlie a  
429 common subtype of adrenal hypertension. *Nat Genet* 45 (9), 1055-60.
- 430 22. Scholl, U.I. et al. (2013) Somatic and germline CACNA1D calcium channel mutations in  
431 aldosterone-producing adenomas and primary aldosteronism. *Nat Genet* 45 (9), 1050-4.
- 432 23. Beuschlein, F. et al. (2013) Somatic mutations in ATP1A1 and ATP2B3 lead to aldosterone-  
433 producing adenomas and secondary hypertension. *Nat Genet* 45 (4), 440-4, 444e1-2.
- 434 24. Fernandes-Rosa, F.L. et al. (2014) Genetic spectrum and clinical correlates of somatic  
435 mutations in aldosterone-producing adenoma. *Hypertension* 64 (2), 354-61.
- 436 25. Akerstrom, T. et al. (2015) Novel somatic mutations and distinct molecular signature in  
437 aldosterone-producing adenomas. *Endocr Relat Cancer* 22 (5), 735-44.
- 438 26. Nanba, K. et al. (2018) Targeted Molecular Characterization of Aldosterone-Producing  
439 Adenomas in White Americans. *J Clin Endocrinol Metab* 103 (10), 3869-3876.
- 440 27. Nanba, K. et al. (2019) Genetic Characteristics of Aldosterone-Producing Adenomas in  
441 Blacks. *Hypertension* 73 (4), 885-892.
- 442 28. De Sousa, K. et al. (2020) Genetic, Cellular, and Molecular Heterogeneity in Adrenals With  
443 Aldosterone-Producing Adenoma. *Hypertension*, HYPERTENSIONAHA11914177.
- 444 29. Scholl, U.I. et al. (2015) Recurrent gain of function mutation in calcium channel CACNA1H  
445 causes early-onset hypertension with primary aldosteronism. *Elife* 4, e06315.

- 446 30. Daniil, G. et al. (2016) CACNA1H Mutations Are Associated With Different Forms of  
447 Primary Aldosteronism. *EBioMedicine* 13, 225-236.
- 448 31. Fernandes-Rosa, F.L. et al. (2018) A gain-of-function mutation in the CLCN2 chloride  
449 channel gene causes primary aldosteronism. *Nat Genet* 50 (3), 355-361.
- 450 32. Scholl, U.I. et al. (2018) CLCN2 chloride channel mutations in familial hyperaldosteronism  
451 type II. *Nat Genet* 50 (3), 349-354.
- 452 33. Oki, K. et al. (2012) The potassium channel, Kir3.4 participates in angiotensin II-stimulated  
453 aldosterone production by a human adrenocortical cell line. *Endocrinology* 153 (9), 4328-35.
- 454 34. Murthy, M. et al. (2012) Characterization of a novel somatic KCNJ5 mutation delI157 in  
455 an aldosterone-producing adenoma. *J Hypertens* 30 (9), 1827-33.
- 456 35. Cheng, C.J. et al. (2015) Novel KCNJ5 mutations in sporadic aldosterone-producing  
457 adenoma reduce Kir3.4 membrane abundance. *J Clin Endocrinol Metab* 100 (1), E155-63.
- 458 36. Yang, Y. et al. (2019) Primary Aldosteronism: KCNJ5 Mutations and Adrenocortical Cell  
459 Growth. *Hypertension* 74 (4), 809-816.
- 460 37. Azizan, E.A. et al. (2012) Somatic mutations affecting the selectivity filter of KCNJ5 are  
461 frequent in 2 large unselected collections of adrenal aldosteronomas. *Hypertension* 59 (3), 587-  
462 91.
- 463 38. Lenzini, L. et al. (2015) A Meta-Analysis of Somatic KCNJ5 K(+) Channel Mutations In  
464 1636 Patients With an Aldosterone-Producing Adenoma. *J Clin Endocrinol Metab* 100 (8),  
465 E1089-95.
- 466 39. Taguchi, R. et al. (2012) Expression and mutations of KCNJ5 mRNA in Japanese patients  
467 with aldosterone-producing adenomas. *J Clin Endocrinol Metab* 97 (4), 1311-9.
- 468 40. Zheng, F.F. et al. (2015) Clinical characteristics of somatic mutations in Chinese patients  
469 with aldosterone-producing adenoma. *Hypertension* 65 (3), 622-8.
- 470 41. Wang, B. et al. (2015) Prevalence and characterization of somatic mutations in Chinese  
471 aldosterone-producing adenoma patients. *Medicine (Baltimore)* 94 (16), e708.
- 472 42. Boulkroun, S. et al. (2012) Prevalence, clinical, and molecular correlates of KCNJ5  
473 mutations in primary aldosteronism. *Hypertension* 59 (3), 592-8.
- 474 43. Scholl, U.I. et al. (2015) Novel somatic mutations in primary hyperaldosteronism are related  
475 to the clinical, radiological and pathological phenotype. *Clin Endocrinol (Oxf)* 83 (6), 779-89.
- 476 44. Vilela, L.A.P. et al. (2019) KCNJ5 somatic mutation is a predictor of hypertension  
477 remission after adrenalectomy for unilateral primary aldosteronism. *J Clin Endocrinol Metab*  
478 104 (10), 4695-4702.

- 479 45. Yamazaki, Y. et al. (2017) Histopathological Classification of Cross-Sectional Image-  
480 Negative Hyperaldosteronism. *J Clin Endocrinol Metab* 102 (4), 1182-1192.
- 481 46. Boulkroun, S. et al. (2013) KCNJ5 mutations in aldosterone producing adenoma and  
482 relationship with adrenal cortex remodeling. *Mol Cell Endocrinol* 371 (1-2), 221-7.
- 483 47. Fernandes-Rosa, F.L. et al. (2015) Functional histopathological markers of aldosterone  
484 producing adenoma and somatic KCNJ5 mutations. *Mol Cell Endocrinol* 408, 220-6.
- 485 48. Geller, D.S. et al. (2008) A novel form of human mendelian hypertension featuring  
486 nonglucocorticoid-remediable aldosteronism. *J Clin Endocrinol Metab* 93 (8), 3117-23.
- 487 49. Mulatero, P. et al. (2013) Role of KCNJ5 in familial and sporadic primary aldosteronism.  
488 *Nat Rev Endocrinol* 9 (2), 104-12.
- 489 50. Scholl, U.I. et al. (2012) Hypertension with or without adrenal hyperplasia due to different  
490 inherited mutations in the potassium channel KCNJ5. *Proc Natl Acad Sci U S A* 109 (7), 2533-  
491 8.
- 492 51. Mulatero, P. et al. (2012) KCNJ5 mutations in European families with nonglucocorticoid  
493 remediable familial hyperaldosteronism. *Hypertension* 59 (2), 235-40.
- 494 52. Monticone, S. et al. (2013) a Novel Y152C KCNJ5 mutation responsible for familial  
495 hyperaldosteronism type III. *J Clin Endocrinol Metab* 98 (11), E1861-5.
- 496 53. Adachi, M. et al. (2014) Discordant Genotype-Phenotype Correlation in Familial  
497 Hyperaldosteronism Type III with KCNJ5 Gene Mutation: A Patient Report and Review of the  
498 Literature. *Horm Res Paediatr* 82 (2), 138-42.
- 499 54. Tauber, P. et al. (2014) Pharmacology and pathophysiology of mutated KCNJ5 found in  
500 adrenal aldosterone producing adenomas. *Endocrinology*, en20131944.
- 501 55. Scholl, U.I. et al. (2017) Macrolides selectively inhibit mutant KCNJ5 potassium channels  
502 that cause aldosterone-producing adenoma. *J Clin Invest* 127 (7), 2739-2750.
- 503 56. Caroccia, B. et al. (2017) Macrolides Blunt Aldosterone Biosynthesis: A Proof-of-Concept  
504 Study in KCNJ5 Mutated Adenoma Cells Ex Vivo. *Hypertension* 70 (6), 1238-1242.
- 505 57. Maiolino, G. et al. (2018) Macrolides for KCNJ5-mutated aldosterone-producing adenoma  
506 (MAPA): design of a study for personalized diagnosis of primary aldosteronism. *Blood Press*  
507 27 (4), 200-205.
- 508 58. Nanba, K. et al. (2020) Somatic CACNA1H Mutation As a Cause of Aldosterone-Producing  
509 Adenoma. *Hypertension* 75 (3), 645-649.
- 510 59. Williams, T.A. et al. (2014) Somatic ATP1A1, ATP2B3, and KCNJ5 mutations in  
511 aldosterone-producing adenomas. *Hypertension* 63 (1), 188-95.

512 60. Stindl, J. et al. (2015) Pathogenesis of Adrenal Aldosterone-Producing Adenomas Carrying  
513 Mutations of the Na(+)/K(+)-ATPase. *Endocrinology* 156 (12), 4582-91.

514 61. Li, C. et al. (2005) A third Na<sup>+</sup>-binding site in the sodium pump. *Proc Natl Acad Sci U S*  
515 *A* 102 (36), 12706-11.

516 62. Dutta, R.K. et al. (2014) Complementary somatic mutations of KCNJ5, ATP1A1, and  
517 ATP2B3 in sporadic aldosterone producing adrenal adenomas. *Endocr Relat Cancer* 21 (1), L1-  
518 4.

519 63. Tauber, P. et al. (2016) Cellular Pathophysiology of an Adrenal Adenoma-Associated  
520 Mutant of the Plasma Membrane Ca<sup>2+</sup>-ATPase ATP2B3. *Endocrinology* 157 (6), 2489-99.

521 64. Stowasser, M. et al. (1992) Familial hyperaldosteronism type II: five families with a new  
522 variety of primary aldosteronism. *Clin Exp Pharmacol Physiol* 19 (5), 319-22.

523 65. Stowasser, M. and Gordon, R.D. (2001) Familial hyperaldosteronism. *J Steroid Biochem*  
524 *Mol Biol* 78 (3), 215-29.

525 66. Dutta, R.K. et al. (2019) A somatic mutation in CLCN2 identified in a sporadic aldosterone-  
526 producing adenoma. *Eur J Endocrinol* 181 (5), K37-K41.

527 67. Akerstrom, T. et al. (2016) Activating mutations in CTNNB1 in aldosterone producing  
528 adenomas. *Sci Rep* 6, 19546.

529 68. Teo, A.E. et al. (2015) Pregnancy, Primary Aldosteronism, and Adrenal CTNNB1  
530 Mutations. *N Engl J Med* 373 (15), 1429-36.

531 69. Gagnon, N. et al. (2018) Genetic Characterization of GnRH/LH-Responsive Primary  
532 Aldosteronism. *J Clin Endocrinol Metab* 103 (8), 2926-2935.

533 70. Rhayem, Y. et al. (2016) PRKACA Somatic Mutations Are Rare Findings in Aldosterone-  
534 Producing Adenomas. *J Clin Endocrinol Metab* 101 (8), 3010-7.

535 71. Zilbermint, M. et al. (2015) Primary Aldosteronism and ARMC5 Variants. *J Clin*  
536 *Endocrinol Metab* 100 (6), E900-9.

537 72. Mulatero, P. et al. (2016) ARMC5 mutation analysis in patients with primary aldosteronism  
538 and bilateral adrenal lesions. *J Hum Hypertens* 30 (6), 374-8.

539 73. Assie, G. et al. (2013) ARMC5 mutations in macronodular adrenal hyperplasia with  
540 Cushing's syndrome. *N Engl J Med* 369 (22), 2105-14.

541 74. Heitzmann, D. et al. (2008) Invalidation of TASK1 potassium channels disrupts adrenal  
542 gland zonation and mineralocorticoid homeostasis. *EMBO J* 27 (1), 179-87.

543 75. Davies, L.A. et al. (2008) TASK channel deletion in mice causes primary  
544 hyperaldosteronism. *Proc Natl Acad Sci U S A* 105 (6), 2203-8.

545 76. Penton, D. et al. (2012) Task3 potassium channel gene invalidation causes low renin and  
546 salt-sensitive arterial hypertension. *Endocrinology* 153 (10), 4740-8.

547 77. Guagliardo, N.A. et al. (2012) TASK-3 channel deletion in mice recapitulates low-renin  
548 essential hypertension. *Hypertension* 59 (5), 999-1005.

549 78. Guagliardo, N.A. et al. (2019) Adrenal Tissue-Specific Deletion of TASK Channels Causes  
550 Aldosterone-Driven Angiotensin II-Independent Hypertension. *Hypertension* 73 (2), 407-414.

551 79. Bandulik, S. et al. (2013) Severe hyperaldosteronism in neonatal Task3 potassium channel  
552 knockout mice is associated with activation of the intraadrenal renin-angiotensin system.  
553 *Endocrinology* 154 (8), 2712-22.

554 80. El Wakil, A. et al. (2012) Dkk3 is a component of the genetic circuitry regulating  
555 aldosterone biosynthesis in the adrenal cortex. *Hum Mol Genet* 21 (22), 4922-9.

556 81. Goppner, C. et al. (2019) Pathogenesis of hypertension in a mouse model for human CLCN2  
557 related hyperaldosteronism. *Nat Commun* 10 (1), 4678.

558 82. Schewe, J. et al. (2019) Elevated aldosterone and blood pressure in a mouse model of  
559 familial hyperaldosteronism with CIC-2 mutation. *Nat Commun* 10 (1), 5155.

560 83. Billet, S. et al. (2007) Gain-of-function mutant of angiotensin II receptor, type 1A, causes  
561 hypertension and cardiovascular fibrosis in mice. *J Clin Invest* 117 (7), 1914-25.

562 84. Taylor, M.J. et al. (2020) Chemogenetic activation of adrenocortical Gq signaling causes  
563 hyperaldosteronism and disrupts functional zonation. *J Clin Invest* 130 (1), 83-93.

564 85. Tissier, F. et al. (2005) Mutations of beta-catenin in adrenocortical tumors: activation of the  
565 Wnt signaling pathway is a frequent event in both benign and malignant adrenocortical tumors.  
566 *Cancer Res* 65 (17), 7622-7.

567 86. Boulkroun, S. et al. (2011) Aldosterone-producing adenoma formation in the adrenal cortex  
568 involves expression of stem/progenitor cell markers. *Endocrinology* 152 (12), 4753-63.

569 87. Berthon, A. et al. (2014) WNT/beta-catenin signalling is activated in aldosterone-producing  
570 adenomas and controls aldosterone production. *Hum Mol Genet* 23 (4), 889-905.

571 88. Dihazi, G.H. et al. (2015) Proteomic characterization of adrenal gland embryonic  
572 development reveals early initiation of steroid metabolism and reduction of the retinoic acid  
573 pathway. *Proteome Sci* 13, 6.

574 89. El Zein, R.M. et al. (2019) Retinoic acid receptor alpha as a novel contributor to adrenal  
575 cortex structure and function through interactions with Wnt and Vegfa signalling. *Sci Rep* 9  
576 (1), 14677.

577 90. Nishimoto, K. et al. (2010) Adrenocortical zonation in humans under normal and  
578 pathological conditions. *J Clin Endocrinol Metab* 95 (5), 2296-305.

- 579 91. Boulkroun, S. et al. (2010) Adrenal cortex remodeling and functional zona glomerulosa  
580 hyperplasia in primary aldosteronism. *Hypertension* 56 (5), 885-92.
- 581 92. Nishimoto, K. et al. (2015) Aldosterone-stimulating somatic gene mutations are common  
582 in normal adrenal glands. *Proc Natl Acad Sci U S A* 112 (33), E4591-9.
- 583 93. Nishimoto, K. et al. (2017) Immunohistochemistry of aldosterone synthase leads the way  
584 to the pathogenesis of primary aldosteronism. *Mol Cell Endocrinol* 441, 124-133.
- 585 94. Omata, K. et al. (2018) Cellular and Genetic Causes of Idiopathic Hyperaldosteronism.  
586 *Hypertension* 72 (4), 874-880.
- 587 95. Gomez-Sanchez, C.E. and Gomez-Sanchez, E.P. (2012) Mutations of the potassium channel  
588 KCNJ5 causing aldosterone-producing adenomas: one or two hits? *Hypertension* 59 (2), 196-  
589 7.
- 590 96. Fernandes-Rosa, F.L. et al. (2015) Different Somatic Mutations in Multinodular Adrenals  
591 With Aldosterone-Producing Adenoma. *Hypertension* 66 (5), 1014-22.
- 592 97. Vouillarmet, J. et al. (2016) Aldosterone-Producing Adenoma With a Somatic KCNJ5  
593 Mutation Revealing APC-Dependent Familial Adenomatous Polyposis. *J Clin Endocrinol*  
594 *Metab* 101 (11), 3874-3878.

595

596 **Figure legends**

597 **Figure 1. Cellular mechanisms of autonomous aldosterone biosynthesis.** In unstimulated  
598 conditions, zona glomerulosa cells are in a hyperpolarized state, due to the presence of a large  
599 number of potassium channels. Cell membrane depolarization, following stimulation of  
600 aldosterone biosynthesis, leads to opening of voltage-gated calcium channels and activation of  
601 calcium signaling, the main trigger for aldosterone biosynthesis. Mutations in different channels  
602 and pumps have been identified in different forms of familial hyperaldosteronism and APA.  
603 Mutations in GIRK4 potassium channels in APA and FH-III lead to sodium entry into the cells  
604 instead of extrusion of potassium. Mutations in CLC-2 chloride channels in FH-II and APA  
605 increase chloride efflux, and mutations in the  $\alpha 1$  subunit of the  $\text{Na}^+, \text{K}^+$ -ATPase, found in APA,  
606 are responsible for loss of pump activity, but also lead to proton entry into the cells. These  
607 mutations lead to cell membrane depolarization and opening of voltage gated calcium channels.  
608 Mutations in the  $\alpha 1$  subunit of the L-type  $\text{Ca}^{2+}$  channel Cav1.3, found in APA and PASNA, and  
609 in  $\alpha 1$  subunit of the T-type  $\text{Ca}^{2+}$  channel Cav3.2 in FH-IV and APA, result in changes in their  
610 functional properties, particularly allowing calcium entry at lower membrane potential, whereas  
611 mutations in PMCA3 are responsible for loss of pump activity and accumulation of calcium in  
612 the cells. All these mutations induce an increase of intracellular calcium concentration and  
613 activation of calcium signaling, followed by stimulation of CYP11B2 transcription and increase  
614 in aldosterone biosynthesis. In FH-I, a chimeric gene in which the coding region of *CYP11B2*  
615 is fused to the regulatory region of *CYP11B1* leads to ectopic expression of *CYP11B2*  
616 throughout the adrenal cortex and control of aldosterone biosynthesis mainly by ACTH.

617

618 **Figure 2: Pathogenic model of aldosterone producing adenoma development.** Two  
619 different models may explain APA development in the adrenal cortex. (Left panel) “APCC  
620 model”: occurrence of somatic mutations in different APA driver genes (*KCNJ5*, *CACNA1D*,  
621 *ATP1A1*, *ATP2B3*, *KCNJ5*) lead to the development of aldosterone producing cell clusters  
622 (APCC). APCC may develop into APA through the formation of possible APCC-to-APA  
623 translational lesions (pAATL). (Right panel) “Two-hit model”: Abnormal cell proliferation and  
624 nodulation in the zona glomerulosa, due to genetic or environmental factors, may create a  
625 propitious environment for the occurrence of somatic mutations in APA driver genes (*KCNJ5*,  
626 *CACNA1D*, *ATP1A1*, *ATP2B3*, *CACNA1H*, *CLCN2*). In both hypothesis, activation of calcium  
627 signaling, due to the presence of somatic mutations, leads to increased *CYP11B2* expression  
628 and autonomous aldosterone production.

629 **Table 1. Familial and inherited forms of primary aldosteronism.**

Disease	Age of onset	Specific features	Gene	Transmission	Treatment
Familial hyperaldosteronism type I	Variable Often before 20 ys	Cerebrovascular events at young age (<30 ys)	Chimeric <i>CYP11B1/B2</i>	AD	Glucocorticoids, MRA
Familial hyperaldosteronism type II	Variable Young onset in patients with <i>CLCN2</i> mutations	none	<i>CLCN2</i>	AD	MRA
Familial hyperaldosteronism type III	Before 20 ys Variable in mild forms	Massive bilateral adrenal hyperplasia in severe cases	<i>KCNJ5</i>	AD	MRA Bilateral adrenalectomy in severe cases
Familial hyperaldosteronism type IV	Variable Most frequent before 20 ys	Developmental disorder in some cases	<i>CACNA1H</i>	AD	MRA
Primary Aldosteronism, Seizures and Neurologic Abnormalities (PASNA)	Childhood	Seizures and neurological abnormalities	<i>CACNA1D</i>	? (de novo)	Calcium channel blocker

**AD:** autosomal dominant; MRA: Mineralocorticoid Receptor antagonist

**Table 2. Recent mouse models of hyperaldosteronism.**

Model	Phenotype	Adrenal Phenotype	Mechanism	Ref
TASK1 inactivation	<ul style="list-style-type: none"> <li>• Hyperaldosteronism remediable by glucocorticoids</li> <li>• Both sexes in young mice</li> <li>• Restricted to females in adult mice</li> </ul>	<ul style="list-style-type: none"> <li>• Cyp11B2 absent in the ZG</li> <li>• Ectopic Cyp11B2 expression in the ZF restricted to females in adults.</li> </ul>	<ul style="list-style-type: none"> <li>• More depolarized membrane voltage at resting conditions</li> </ul>	[74]
TASK1 and TASK3 inactivation	<ul style="list-style-type: none"> <li>• Overproduction of aldosterone resistant to salt suppression</li> <li>• Normal/low renin</li> </ul>	<ul style="list-style-type: none"> <li>• Cellular morphology and zonation preserved in adult mice (only males)</li> </ul>	<ul style="list-style-type: none"> <li>• Marked depolarization of ZG cells.</li> </ul>	[75]
TASK3 inactivation	<ul style="list-style-type: none"> <li>• Salt-sensitive, low renin, arterial hypertension</li> <li>• Abnormalities in the regulation of aldosterone</li> <li>• Severe hyperaldosteronism in neonatal knockout mice</li> </ul>	<ul style="list-style-type: none"> <li>• No changes in adrenal morphology</li> </ul>	<ul style="list-style-type: none"> <li>• Depolarization of adrenocortical cells.</li> <li>• Abnormal Ca<sup>2+</sup> signaling.</li> </ul>	[76, 79]
TASK3 inactivation	<ul style="list-style-type: none"> <li>• Salt-sensitive, low renin, arterial hypertension</li> <li>• Mild hyperaldosteronism resistant to salt suppression</li> </ul>	<ul style="list-style-type: none"> <li>• No changes in adrenal morphology</li> </ul>	<ul style="list-style-type: none"> <li>• More depolarized membrane voltage at resting conditions</li> </ul>	[77]
TASK1 and DKK3 inactivation	<ul style="list-style-type: none"> <li>• Hyperaldosteronism remediable by glucocorticoids in both sexes</li> </ul>	<ul style="list-style-type: none"> <li>• Absence of altered functional zonation in male mice</li> </ul>	<ul style="list-style-type: none"> <li>• More depolarized membrane voltage at resting conditions</li> </ul>	[80]
Constitutive activation of $\beta$ -catenin	<ul style="list-style-type: none"> <li>• Increased aldosterone production independent of renin</li> </ul>	<ul style="list-style-type: none"> <li>• Ectopic expression of Dab2, Beta-catenin and Cyp11b2 in ZF</li> </ul>	<ul style="list-style-type: none"> <li>• Beta-catenin dependent activation of <i>at1r</i> and <i>cyp21</i></li> <li>• Increased expression of Nurr1 and Nur77 leading to <i>Cyp11b2</i> activation</li> </ul>	[87]
ZG Specific inactivation of TASK1 and TASK3	<ul style="list-style-type: none"> <li>• Arterial hypertension</li> <li>• Mild hyperaldosteronism</li> <li>• Mild aldosterone autonomy</li> </ul>	NA	NA	[78]

Constitutively open CIC-2	<ul style="list-style-type: none"> <li>• Marked hypertension</li> <li>• High serum aldosterone</li> <li>• Low renin activity</li> <li>• Hypokalemia</li> </ul>	<ul style="list-style-type: none"> <li>• No changes in adrenal morphology, increased expression of <i>Cyp11b2</i> in the ZG</li> </ul>	<ul style="list-style-type: none"> <li>• Increased chloride conductance in the ZG [81]</li> <li>• Cell membrane depolarization</li> <li>• Increased cytoplasmic <math>Ca^{2+}</math> concentration</li> <li>• Increased <i>Cyp11b2</i> expression</li> </ul>
Knock-in <i>Clcn2</i> model (homologous to human <i>CLCN2</i> mutation in FH-II)	<ul style="list-style-type: none"> <li>• Mildly elevated blood pressure</li> <li>• Mildly elevated aldosterone levels</li> <li>•</li> </ul>	<ul style="list-style-type: none"> <li>• No changes in adrenal morphology</li> <li>• Incomplete suppression of <i>Cyp11b2</i> expression upon high-salt challenge</li> </ul>	<ul style="list-style-type: none"> <li>• Increased calcium oscillatory activity in adrenal cells [82]</li> <li>• Increased <i>Cyp11b2</i> expression</li> </ul>
Specific and inducible adrenal activation of Gq signaling	<ul style="list-style-type: none"> <li>• Hypertension</li> <li>• Increased aldosterone levels</li> <li>• Decreased renin</li> </ul>	<ul style="list-style-type: none"> <li>• Disorganization of adrenal functional zonation</li> </ul>	<ul style="list-style-type: none"> <li>• Gq signaling dependent <i>Cyp11b2</i> expression in ZF cells [84]</li> </ul>

---

ZG: zona glomerulosa; ZF: zona fasciculata; NA: not available

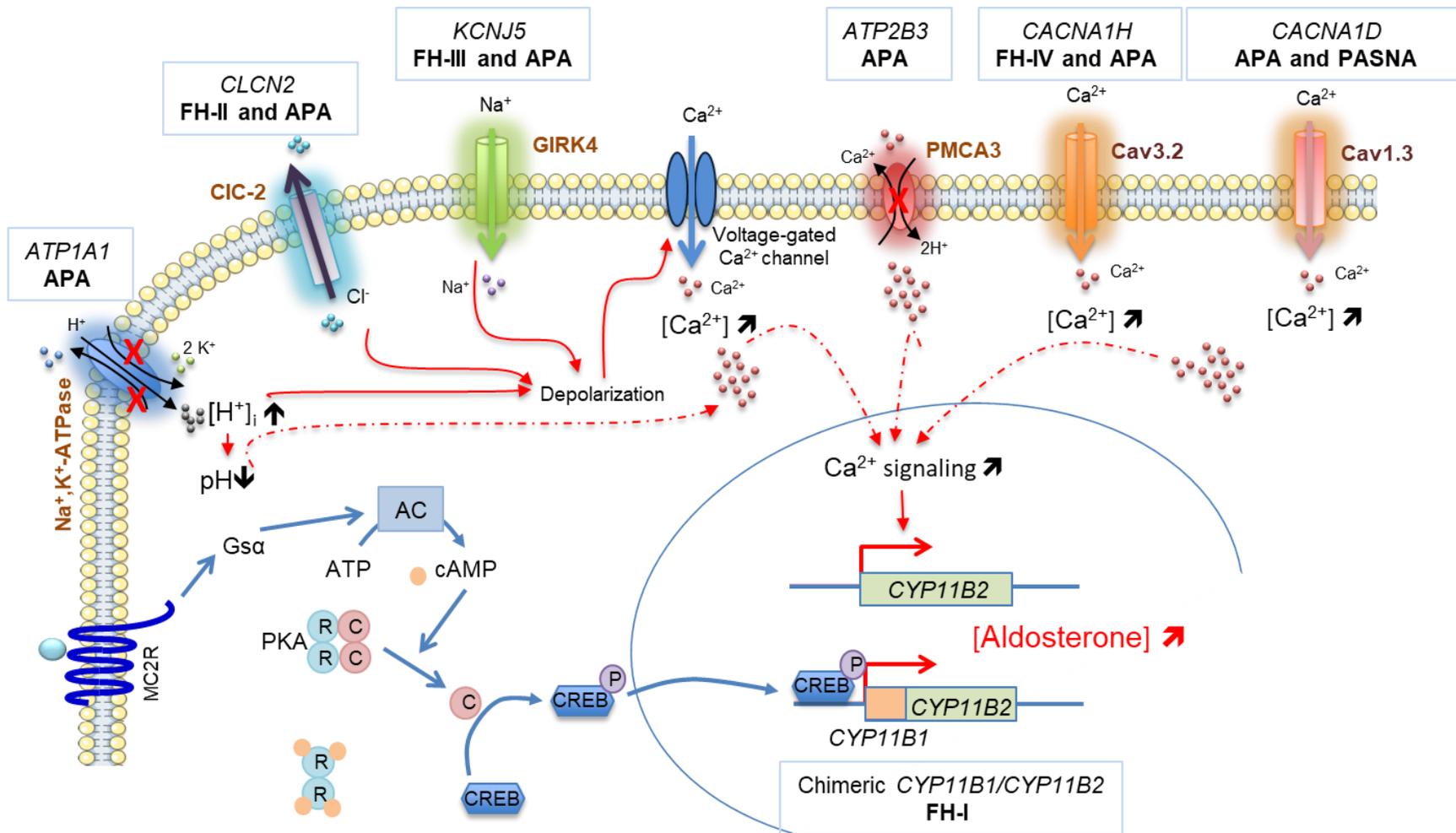


Figure 1

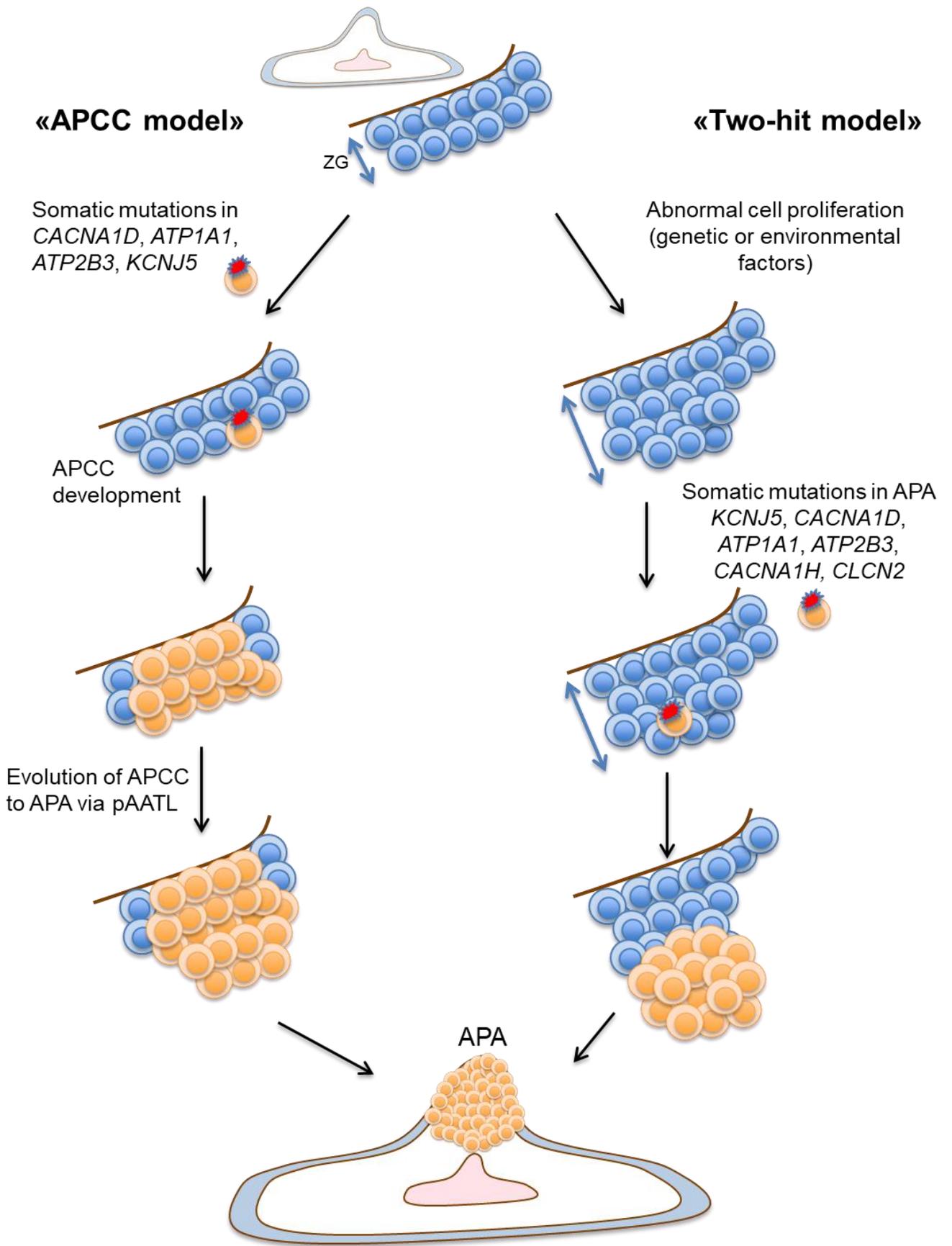


Figure 2

