

Inherited Forms of Primary Hyperaldosteronism: New Genes, New Phenotypes and Proposition of A New Classification

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ABSTRACT

Primary aldosteronism is a common cause of endocrine hypertension. It results from the excess production of aldosterone by the adrenal cortex and is related to increased morbidity and mortality. Most cases of PA are sporadic but inherited patterns of the disease have been reported in the literature. Four forms of familial hyperaldosteronism (FH-I- FH-IV) are currently recognized, and the genetic basis has been clarified in recent years. In FH-I patients, aldosterone excess is produced by a CYP11B1/ CYP11B2 fusion gene and it is suppressed by glucocorticoid treatment. FH-II is caused by mutations in the inwardly rectifying chloride channel CLCN2. FH-III is caused by mutations in KCNJ5, a gene coding for an inward rectifier K⁺ channel and mutations in the T-type calcium channel subunit CACNA1H cause FH-IV. In this review we summarize the knowledge on inherited forms of primary aldosteronism, the genetic alterations that cause them and the implications it may have for the classification. Based on current evidence, we propose the term “familial hyperaldosteronism” to refer only to inherited forms of primary aldosteronism with a known genetic basis.

Abbreviations

PA	primary aldosteronism
FH	familial hyperaldosteronism
APA	aldosterone-producing adenoma
MR	mineralocorticoid receptor.

Primary aldosteronism (PA) is the clinical manifestation of a heterogeneous group of adrenal disorders that are characterized by an excessive production of aldosterone, which becomes relatively independent of the angiotensin-renin system regulation. Over time, sustained levels of aldosterone lead to increased blood pressure and elevated potassium excretion, therefore patients with PA are hypertensive, in many cases hypokalemic, and at higher risk of stroke, renal complications, metabolic and cardiovascular mortal-

ity than patients with essential hypertension. Once classified as a rare disease, PA is now considered the most common cause of endocrine hypertension, with an estimated prevalence of about 4–6% in the general population with hypertension and up to 10–20% in the subset of patients with resistant hypertension [1–3].

Most diagnosed cases of PA are sporadic and are mainly caused by aldosterone overproduction by both adrenal glands (bilateral adrenal hyperplasia) or by unilateral aldosterone-producing adenomas (APA). Other causes include unilateral hyperplasia and very rarely, adrenocortical carcinomas. In some cases, PA affects several members of the same family in the inherited or familial forms of hyperaldosteronism (FH). Current guidelines recognize three well established types of FH, namely FH-I to FH-III [4], however data from genetic analyses reveal a more complex situation, with at least 4 different inheritable forms of PA and possibly still more yet to be discovered.

Genes Associated With Inherited Forms of PA

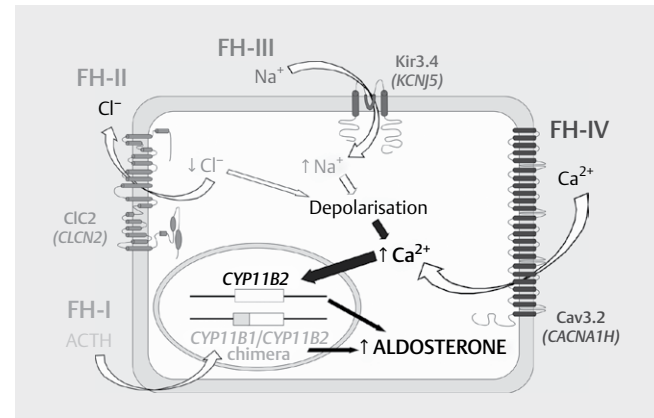
The genetics of PA has remained obscure for a long time. Although infrequent, the early onset and the heritability favored the study of familial PA as an approach to understand the pathophysiology of the more common sporadic forms. The identification of the first genetic alteration causative for a particular subtype of PA by linkage analysis on affected relatives, the chimera CYP11B1/CYP11B2 [5], was an outstanding discovery but subsequent investigation quickly revealed that it was not present in sporadic forms [6, 7]. The failure to find new causative genes and the introduction of next generation sequencing techniques turned the focus to sporadic patients.

Now that hundreds of APAs have been sequenced, it is well known that KCNJ5, CACNA1D, ATP1A1 and ATP2B3 genes are mutated in about 50% of adenomas (reviewed in [8] and [9]) and that ion channels and pumps exert an important role on aldosterone signaling through the control of Ca^{2+} influx [10]. Following the trend of next generation sequencing of sporadic cases, the study of patients with early-onset PA has uncovered that some of those genes also exert an important role in inherited forms. Thus, KCNJ5 germline mutations cause FH-III, CACNA1H mutations have been found in families with FH-IV and de novo germline mutations in CACNA1D have been reported in patients with early onset of PA, seizures and neurologic abnormalities (PASNA). In addition, two recent studies in patients with early-onset PA have shown mutations in CLCN2 associated with FH-II. ▶ **Table 1** summarizes the genes associated with PA and the main clinical features and ▶ **Fig. 1** depicts the molecular mechanisms.

CYP11B1/CYP11B2 chimera: familial hyperaldosteronism type I (FH-I)

FH-I was first reported in 1966 by Sutherland and colleagues [11]. They reported two hypertensive relatives, a father and a son, with a condition that mimicked the symptoms of sporadic PA (increased aldosterone, low renin activity and hypokalemia) but with the particularity of suppression of aldosterone production by a 2 mg/day dexamethasone treatment. Thus FH-I is often referred to as gluco-

corticoid-remediable aldosteronism (GRA). The basis of the glucocorticoid suppression was discovered in 1992, after linkage analysis identified the genetic cause as a chimeric fusion on chromosome 8 containing an unequal recombination between the highly homologous genes CYP11B1 (11- β -hydroxylase) and CYP11B2 (aldosterone synthase) [5]. The exact point of cross-over can be different in each reported family but always contains the promoter and the first exons of CYP11B1 and most of the coding region of CYP11B2, resulting in an enzyme with aldosterone synthase activity with



▶ **Fig. 1** Genetic causes of familial hyperaldosteronism. Summary of the known molecular mechanisms that lead to familial hyperaldosteronism types I to IV. FH-I is produced by an asymmetrical recombination between CYP11B1 and CYP11B2, resulting in the expression of a chimeric enzyme under the control of ACTH stimulation. FH-II is caused by germline mutations in the chloride channel CLCN2 that decrease intracellular Cl^- . FH-III is produced by germline mutations in KCNJ5 that affect the selectivity of the channel, allowing Na^+ conductance. Both reduction of intracellular Cl^- and increase in Na^+ cause plasma membrane depolarization and open voltage-gated Ca^{2+} channels, elevating cytosolic Ca^{2+} . FH-IV is caused by germline mutations in CACNA1H that facilitate Ca^{2+} entry. In all cases, increase of intracellular Ca^{2+} triggers CYP11B2 transcription and aldosterone synthesis.

▶ **Table 1** Germline mutations associated with primary aldosteronism.

Germline alteration	Biological effect	Clinical manifestation	Treatment	Pattern of heritability
CYP11B1/CYP11B2 chimeric fusions	Aldosterone synthase inducible by ACTH stimulation	Glucocorticoid-suppressible hyperaldosteronism (FH-I)	Low dose of dexamethasone, MR antagonists as second line	Autosomal dominant
CLCN2 mutations	Increased Cl^- efflux	FH-II	MR antagonists	Autosomal dominant, incomplete penetrance
KCNJ5 mutations	Reduced K^+ selectivity and increased Na^+ influx	FH-III	MR antagonist if mild, adrenalectomy if severe	Autosomal dominant
CACNA1H mutations	Increased Ca^{2+} entry	FH-IV	Not standardized. No control observed under spironolactone treatment or unilateral adrenalectomy	Autosomal dominant, low penetrance
CACNA1D mutations	Increased Ca^{2+} entry	PA associated with seizures and neurological abnormalities (PASNA)	MR antagonist, adrenalectomy if lateralized	Autosomal dominant not likely to be inherited
ARMC5 mutations	Loss of ARMC5 expression/function	Apparently sporadic, possible predisposition to PA	Data not available	Autosomal dominant, incomplete penetrance

PA, primary aldosteronism; FH, Familial hyperaldosteronism; MR, mineralocorticoid receptor

expression under the control of the adrenocorticotrophic hormone (ACTH) instead of angiotensin II and potassium. As a consequence, aldosterone synthase is expressed in the zona fasciculata rather than in the zona glomerulosa, resulting in the ectopic production of aldosterone and the production of the hybrid steroids 18-oxocortisol and 18-hydroxycortisol [5, 12].

FH-I is considered as a rare subgroup of PA that represents less than 1 % of all cases, increasing to 3 % in children with hypertension [13–15]. It is characterized by the development of bilateral adrenal hyperplasia, occasionally adrenal nodules, with variable clinical and biochemical features [14, 16]. FH-I follows an autosomal dominant inheritance pattern and is generally associated with early onset severe hypertension and an increased risk of stroke; however, different degrees of severity have been reported, including cases of mild hypertension and normotensive individuals [16–18].

The Endocrine Society guideline recommends testing for FH-I in patients with an early onset of PA (<20 years old) and in those with a familial occurrence of PA or stroke at a young age (<40 years old) [4]. The correct diagnosis is clinically relevant because aldosterone excess can be controlled successfully through glucocorticoid therapy [19]. Prior to the existence of targeted molecular tests, the diagnosis was made through clinical and biochemical evaluation. Dexamethasone suppression of aldosterone and levels of hybrid steroids were used to establish a diagnosis of FH-I [20, 21] until the introduction of techniques to specifically detect the presence of the CYP11B1/CYP11B2 chimeric gene either by Southern blotting or by the recommended technique employing a long-chain PCR amplification [4, 21–23].

In patients with FH-I aldosterone production is abrogated under glucocorticoid treatment, and partial suppression of ACTH is enough to correct the hypertension associated with FH-I. Accordingly, low doses of dexamethasone are recommended to achieve normotension whilst preventing undesired cushingoid features [19]. Mineralocorticoid receptor antagonists (spironolactone or eplerenone) can be used as a second line of therapy to block possible non-genomic effects of aldosterone on target organs, or in children to avoid possible side effects of dexamethasone treatment [24]. If target blood pressure levels are not reached by low dose dexamethasone, additional standard antihypertensive medication can be added (i. e. calcium antagonists, beta-blockers).

CLCN2: familial hyperaldosteronism type II (FH-II)

FH-II was first described by Gordon et al. in 1991, a year before the genetic cause of FH-I was published. They described 6 relatives from 3 independent affected families who presented with PA caused by either APA or BAH and a lack of suppression of aldosterone production by fludrocortisone or dexamethasone [25]. Several families were reported by the same group shortly thereafter [26, 27].

Until very recently, the genetic cause of FH-II remained elusive. Early targeted genetic studies showed a lack of mutations on genes related to steroidogenic production or tumorigenesis, such as CYP11B2, the angiotensin receptor AT1R or TP53. Later on, genetic linkage analysis of non-related families highlighted a locus at chromosome 7p22 that segregated with the disease in some families, but not in all cohorts [28–30]. However both targeted sequencing of different genes in that region as well as next-genera-

tion sequencing of the complete locus failed to find mutations [29, 31–33]. Scholl and colleagues analyzed the genomic DNA by exome sequencing of three members from one of the FH-II families described by Stowasser et al. in 1992 [27]. The authors identified a germline mutation in the gene CLCN2 that segregated with the disease. This variant, p.Arg172Gln, was confirmed subsequently in five additional family members, four of them with aldosterone-to-renin ratio suggestive of PA [34]. Because the discovery family was one of the first families diagnosed with FH-II, Scholl et al. proposed the use of that term only for inherited PA due to CLCN2 mutations [34]. The authors also reported the same mutation in three additional unrelated individuals, as well as rare germline CLCN2 variants (p.Met22Lys, p.Tyr26Asn, p.Lys362del and Ser865Arg, with allele frequencies below 10^{-5}) in four additional unrelated patients [34]. Simultaneously, Fernandes-Rosa and colleagues identified another germline CLCN2 mutation in a 9-year-old patient by exome-sequencing sequencing of genomic DNA from 12 patients with young-onset hypertension and PA. In that case, p.Gly24Asp was a de novo mutation. Two additional variants were found in two cases from a cohort of 100 patients with idiopathic bilateral adrenal hyperplasia (p.Arg66Gln and p.Pro48Arg, with minor allele frequencies of 3×10^{-5} and 1.7×10^{-4} , respectively) [35]. Both studies showed that CLCN2 mutations were related to PA diagnosed at early age and absent in patients with essential hypertension [34, 35].

CLCN2 gene is located in chromosome 3q27 and encodes the inwardly rectifying chloride channel CIC2, a member of the CLC voltage-gated Cl^- channels family. CIC2 is broadly expressed in mammalian cells, especially in brain, gut, kidney, heart and liver [36]. Mutations inactivating CLCN2 cause leukodystrophy, in some cases with azoospermia, and Clcn2 knockout mice also develop early postnatal retinal degeneration [37–39]. Scholl et al. and Fernandes-Rosa et al. have shown that CIC2 is also expressed in the adrenal gland. Furthermore, germline mutations that associate with PA result in gain of function of the Cl^- channel, causing an efflux of Cl^- ions that leads to the depolarization of the plasma membrane, the consequent opening of voltage-gated Ca^{2+} channels, the accumulation of cytosolic Ca^{2+} and the activation of CYP11B2 transcription [34, 35].

Before the recent discovery of CLCN2 mutations, screening for FH-II was based on the diagnosis of PA in at least two first-degree members of the same family and the absence of known germline mutations. Thus, this familial form was thought to be the most prevalent, representing about 3–6 % of all PA cases [11, 54]. Nevertheless, Korah and Scholl pointed out that this estimation may be misleading: considering the prevalence of hypertension in the general population (about 30 %) and the PA prevalence in the general population with hypertension (about 5 %), the probability for an index case to have at least a first-degree relative with PA just by chance is ~5.9 % [40]. Accordingly, it is likely that some of the described FH-II families were in fact coincidental cases of sporadic idiopathic PA. This observation may explain, at least partially, the apparent heterogeneity reported in previous studies. To avoid confusion, and to base the classification on a simple and transparent genetic basis similar to other genetic diseases, we propose to use the term “familial hyperaldosteronism” only when an inherited genetic cause is established.

Following this reasoning, the number of true FH-II families is probably much lower than previously reported. In their studies, Scholl et al. and Fernandes-Rosa et al. identified *CLCN2* mutations in about 10 % of cases with young-onset PA without known germline mutations and 2 % with bilateral adrenal hyperplasia [34, 35] suggesting a lower frequency than previous estimates. Further efforts are needed to determine the actual prevalence of FH-II.

KCNJ5: familial hyperaldosteronism type III (FH-III)

FH-III was described by Geller et al. in three family members, a father and his two young daughters, who developed hyperaldosteronism with hypokalemia and severe hypertension at very early age, together with marked bilateral adrenal enlargement. High levels of the hybrid steroids 18-oxocortisol and 18-hydroxycortisol were detected in urine samples but the disorder was distinguishable from FH-I by the glucocorticoid resistance of the hyperaldosteronism and the lack of suppression of aldosterone production on dexamethasone suppression testing. Hypertension and hypokalemia were refractory to medical therapy and disease control was achieved only after bilateral adrenalectomy [41]. Careful examination of the adrenals revealed disorganized zonation, a reduction in the thickness of the zona glomerulosa, an enlarged zona fasciculata and the presence of cells that co-express enzymes which are usually expressed in distinct zones, such as *CYP11B1* and *CYP11B2* and also *CYP17* and *CYP11B2*. The co-expression of *CYP17* and *CYP11B2* is the likely basis for the production of hybrid steroids [12, 41, 42].

It was not until 2011 that the genetic etiology of FH-III was clarified. By means of exome sequencing, Choi et al. identified a heterozygous germline mutation located on chromosome 11q24 in the patients reported by Geller and colleagues, as well as in sporadic cases of PA [43]. The affected gene was *KCNJ5*, which codes for the G-protein-activated inward rectifier K^+ channel 4 (*Kir3.4*). This protein forms homo- and heterotetramers with other Kir family members to constitute the functional G-protein-activated inwardly rectifying potassium channel, which contributes to the control of membrane polarity in the zona glomerulosa [44]. The mutation identified in Geller's cases (p.Thr158Ala) was associated with a loss in K^+ selectivity and an increased influx of Na^+ into the cytoplasm, leading to membrane depolarization and the elevation of intracellular Ca^{2+} levels, which ultimately triggers aldosterone production through the activation of Ca^{2+} -related signaling pathways [45].

Since the link between inherited PA and *KCNJ5*, several familial cases with different mutations in that gene have been published, mostly in or next to the selectivity filter [46–51], and the term FH-III is used for familial cases with PA due to germline *KCNJ5* mutations, regardless of the phenotype. Indeed, the clinical features of the affected cases vary all along the PA spectrum, from mild and treatment-responding forms to severe PA with progressive disease, including symptoms mimicking diabetes insipidus and a recent report showing development of Cushing's syndrome in one patient with FH-III [50]. This variability seems to be dependent on the type of the grounding *KCNJ5* mutations, among other factors [46]. Thus, p.Gly151Glu mutations seem to associate with a milder phenotype and stable disease [46, 49], while p.Gly151Arg, p.Thr158Ala, p.Ile157Ser and p.Tyr152Cys mutations relate to a more severe hyperaldosteronism [52]. Other infrequent germline alterations of *KCNJ5* (some of them de novo) and a rare non-synonymous SNP

(rs7102584) have been described. The mutation p.Glu145Gln affects a salt bridge close to the selectivity filter, while mutations p.Arg52His, p.Glu246Lys, p.Gly247Arg and the SNP Glu282Gln were located elsewhere in the protein [53, 54]. Except of the p.Gly247Arg, those variants altered channel functionality and increased aldosterone production compared with the wild-type protein.

The prevalence of FH-III has not been established systematically but it is estimated to be present in < 1 % of all PA cases [47]. The Endocrine Society guideline recommends testing for FH-III by sequencing peripheral blood for mutations in *KCNJ5* in those patients with a very early onset of PA [4]. Because of the variety of presentations, treatment for FH-III depends on the severity of the disease. Milder cases can be well controlled with spironolactone, while adrenalectomy is currently the best option to treat resistant forms successfully [52].

CACNA1H: familial hyperaldosteronism type IV (FH-IV)

FH-IV was reported by Scholl and coworkers in a cohort of 40 patients diagnosed with PA in early childhood (at age 10 years or below) and without mutations in any common known PA genes. By whole exome sequencing analysis, a recurrent mutation in the gene *CACNA1H* was identified in five unrelated patients, four males and one female [55]. Shortly thereafter Daniil et al. reported the presence of different mutations in the same gene in two unrelated individuals who were diagnosed originally with FH-II, as well as an adult male case with a de novo mutation and an adult female patient with an APA and a germline mutation in the same gene [56]. Patients showed no apparent signs of seizures, cardiac arrhythmia or muscular or neurological alterations that have been commonly linked to other disorders caused by *CACNA1H* germline mutations or by another Ca^{2+} channel subunit, *CACNA1D* [57], although one of the patients was diagnosed with minor mental retardation and multiplex developmental disorder [56]. So far, eight families with FH-IV have been described.

The gene *CACNA1H* is located on chromosome 16 and encodes the T-type (low voltage activated) calcium channel subunit Cav3.2. This protein is expressed in the zona glomerulosa [55, 57] and, as other Cav3 family members, is activated by small depolarizing changes in the membrane potential [58]. Germline *CACNA1H* mutations have been associated with several diseases including epilepsy, autism and amyotrophic lateral sclerosis [59–61]. In their studies, Scholl et al. and Daniil et al. reported six index cases with germline mutations affecting the residue Met1549, four cases with an inherited p.Met1549Val substitution, one with a de novo p.Met1549Val and one with a de novo p.Met1549Ile [55, 56]. This residue is located in the transmembrane segment S6 of the repeat domain III of Cav3.2, forming a conserved methionine-phenylalanine-valine (MFV) tripeptide motif that controls channel inactivation [62]. Functional experiments have demonstrated that mutations in Met1549 result in a decrease in the inactivation of Cav3.2 compared with the wild-type protein. As a consequence, the channel remains open longer with an increase in Ca^{2+} influx, which activates the expression of *CYP11B2* and other steroidogenic genes [55, 56, 63]. Noteworthy, treatment with a T-type calcium channel blocker abrogated the aberrant *CYP11B2* activation and aldosterone production in HAC15 cells overexpressing Cav3.2 p.Met-

1549Val mutant channels, which indicates that drugs of this class could be useful in the treatment of patients with FH-IV [63].

In their study, Daniil et al. reported 3 additional variants: p.Ser196Leu, located in the voltage sensor region on the transmembrane segment S4 of the repeat domain I of Cav3.2, in a male patient and his sister; p.Pro2083Leu, located in the C-terminal cytoplasmic domain, in another index case and his brother; and a de novo p.Val1951Glu, also located in the C-terminal domain, in a patient with an APA (no familial history available). All mutations altered Cav3.2 function and enhanced aldosterone production to a greater or a lesser degree [56].

Although further studies are needed, available data suggests that FH-IV may be a rare form of FH. It follows an autosomal dominant pattern of heritability but with reduced penetrance, particularly in adults. Indeed, some family members with mutations in p.Met1549 were affected with resistant hypertension and PA and others displayed milder or even normotensive phenotype, suggesting that other factors, such as genetic modifiers, somatic mosaicism or the age of the patient, could restrain the gene defect [55]. The type and location of the mutation may also play a role in the pathophysiology of FH-IV, resembling what has been described for KCNJ5 [46]. This fact could also explain the differences on disease presentation among the index cases: some of them were florid cases of PA at their early childhood but without evidence of adrenal hyperplasia; while other patients were diagnosed in their adulthood, nodularity was detected bilaterally in one patient and an APA was diagnosed in another case [55, 56].

Other Germline Mutations Described in Patients With PA

Although not considered established causes of FH, it is worth mentioning that germline mutations in CACNA1D and ARMC5 have been reported in patients with PA.

CACNA1D codes for Cav1.3, an L-type (high-voltage activated) Ca^{2+} channel subunit and is recurrently mutated in about 10% of sporadic APAs. Most sporadic alterations cause gain of function and lead to an increase of Ca^{2+} influx and the consequent overproduction of aldosterone [57]. Recently, Scholl and coworkers identified two de novo mutations in two unrelated cases diagnosed with PASNA (PA associated with seizures and neurological abnormalities) [57]. Although the severe comorbidities of affected individuals make the heritability of PASNA very unlikely, it is tempting to speculate that other CACNA1D mutations that cause a milder phenotype could be involved in a still not described familial form of PA, in the same way that has been proposed for KCNJ5 in FH-III.

ARMC5 encodes an apoptosis regulator that belongs to the armadillo/ β -catenin-like repeat superfamily. Inactivating mutations in ARMC5 have been reported in both sporadic and inherited primary bilateral macronodular hyperplasia, an adrenocortical disease associated with cortisol excess [64–66]. Mutated ARMC5 promotes cell survival and cortisol production in vitro [64, 65]. Interestingly, germline ARMC5 variants have been identified in patients with apparent sporadic cases of PA [67, 68], suggesting a possible inherited predisposition for nodule formation prior to the hormonal-producing phenotype. Nevertheless, the deleterious effect of

those mutations is still quite unclear, as most variants are predicted to be unlikely pathogenic [69]. Thus, further studies must confirm or refuse the possible role of ARMC5 germline mutations in the etiology of PA.

New Genes, New Phenotypes - We Need a New Classification!

In the recent years, our knowledge on inherited forms of PA has progressed substantially [8, 70]. FH classification has evolved from two clinically distinct forms (FH-I and FH-II) described in the previous Endocrine Society guideline [71] to at least four genetically defined types in which patients are grouped based on the presence of causative mutations (FH-I/CYP11B1/B2 chimera, FH-II/CLCN2, FH-III/KCNJ5 and FH-IV/CACNA1H). Despite substantial scientific advances, some questions remain unanswered. Firstly, the clinical heterogeneity within groups of FH related to variable disease presentation and incomplete penetrance suggest a possible modulation of genetic causes by non-genetic factors. This hypothesis could explain why relatives with germline mutations are apparently asymptomatic. Secondly, the prevalence of FH-II and FH-IV families is still uncertain. Evidence suggests that the frequency of CLCN2 and CACNA1H mutations is low. Thus, extensive studies are needed to determine the actual prevalence and the clinical relevance of these subtypes. Lastly, it must be elucidated whether apparent familial cases without known mutations truly follow inherited patterns of PA. Further next-generation sequencing studies will gain insight into the molecular causes of PA and probably contribute to the establishment of new FH types. Misclassification of sporadic PA cases as FH should be avoided. For that reason, we discourage the use of non-genetic criteria for the screening and classification of FH and propose the term “familial hyperaldosteronism” only to be used when known germline mutations are detected.

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Conflict of Interest

No conflict of interest has been declared by the authors.

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