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Pathogenesis of Familial Hyperaldosteronism Type II: New Concepts Involving Anion Channels

Michael Stowasser[1] · Martin Wolley[1] · Aihua Wu[1] · Richard D. Gordon[1] · Julia Schewe[2] · Gabriel Stölting[2] · Ute I. Scholl[2]

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Abstract

Purpose of Review The application of advanced genetic techniques has recently begun to unravel the genetic basis for familial primary aldosteronism type 2 (FH-II).

Recent Findings Whole-exome sequencing in a large family with FH-II revealed a shared rare damaging heterozygous variant in *CLCN2* (chr.3: g.184075850C>T, p.Arg172Gln) in three severely affected members. The gene encodes a chloride channel, ClC-2. A cohort of 80 unrelated individuals diagnosed with early-onset primary aldosteronism was also examined for *CLCN2* mutations finding three further occurrences of p.Arg172Gln mutations and four single cases of other potentially damaging heterozygous mutations for an overall prevalence of 9.9%. A concurrent report also found a different *CLCN2* mutation (p.Gly24Asp) in a single severely affected patient from a cohort of 12 with early-onset PA for a prevalence of 8.3%. Cases of primary aldosteronism associated with *CLCN2* mutations appear to be bilateral and respond well to medical treatment. In the adrenal, ClC-2 has been demonstrated to localize predominantly to the zona glomerulosa (ZG), and functional analysis suggests that mutations in ClC-2 predispose ZG cells to depolarization, thus leading to calcium influx via activation of voltage-gated calcium channels and increased aldosterone production.

Summary Germline *CLCN2* mutations appear to account for a substantial proportion of early-onset primary aldosteronism cases, and genetic testing for mutations in this gene should be considered in appropriate cases.

Keywords Primary aldosteronism · Familial hyperaldosteronism type II · Genetics · Chloride channel · Anion channel · Hypertension

Introduction

In 1966, Sutherland and coworkers first reported the familial occurrence of primary aldosteronism (PA) in a father and son in whom hypertension and aldosterone overproduction were relieved by the administration of glucocorticoids [1•]. Over 25 years elapsed before Lifton and colleagues in 1992 finally elucidated the genetic basis of this familial form of PA, a hybrid gene mutation composed of sequences derived from *CYP11B1* (encoding 11-β-hydroxylase) at its 5′ end fused to sequences derived from *CYP11B2* (encoding aldosterone synthase) at its 3′ end [2••, 3]. Although it took almost 20 more years before additional PA-causing germline mutations were discovered, major progress has occurred since that time, with no fewer than four further genes implicated since 2011. While the first three encode cation channels (*KCNJ5* [4••, 5, 6], *CACNA1H* [7••, 8], and *CACNA1D* [9••]), the last is unique in encoding an anion channel (*CLCN2*) [10•, 11••] (Fig. 1) and is the main subject of the current report.

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✉ Michael Stowasser
m.stowasser@uq.edu.au

[1] Endocrine Hypertension Research Centre, University of Queensland Diamantina Institute, Princess Alexandra Hospital, Ipswich Road, Woolloongabba, Brisbane 4102, Australia

[2] Department of Nephrology and Medical Intensive Care, BCRT, Charité – Universitätsmedizin Berlin and Berlin Institute of Health, Berlin, Germany

Primary Aldosteronism: a Highly Prevalent and Harmful Condition

In PA, production of aldosterone by the adrenal cortex is excessive for the body's prevailing sodium/volume status

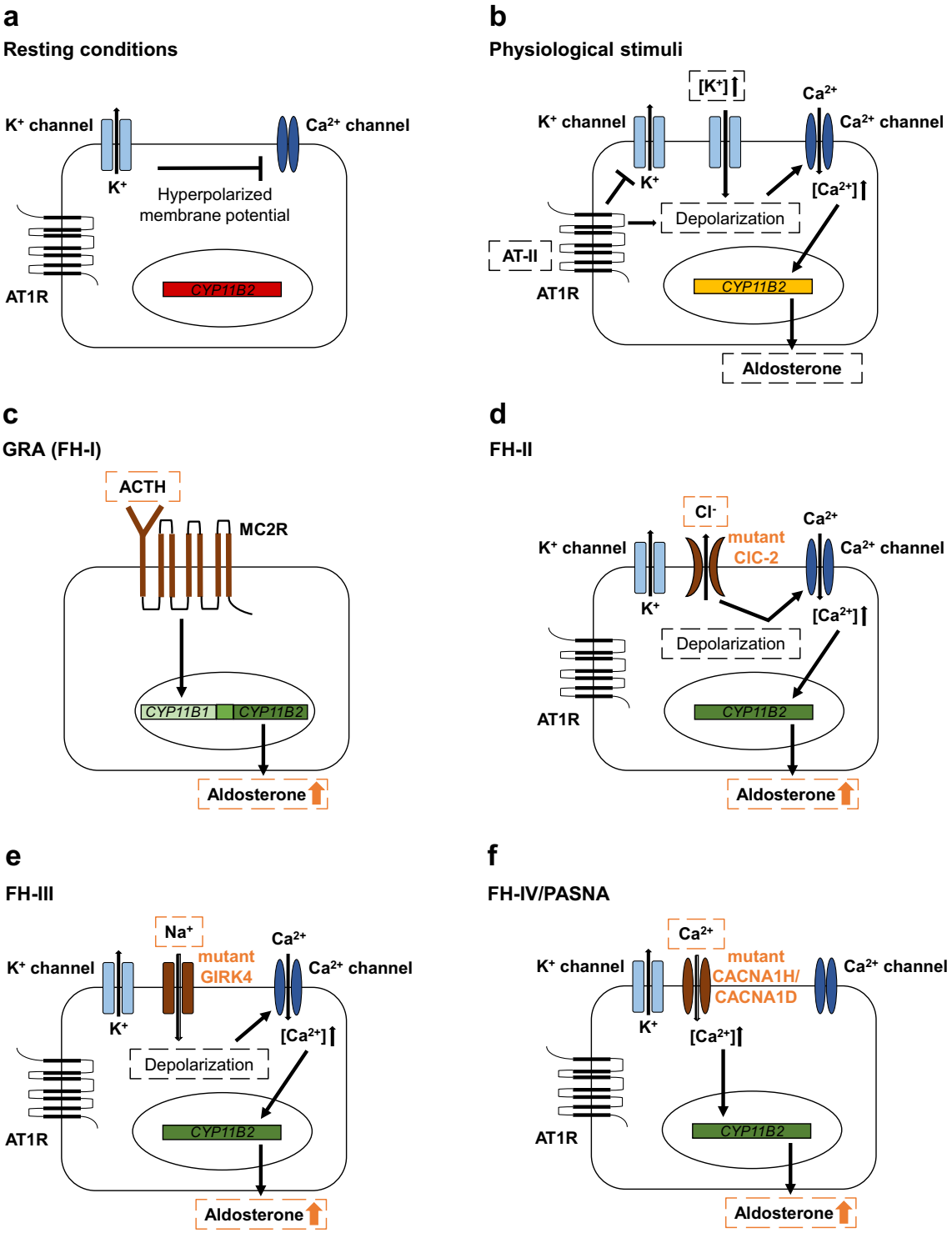

Fig. 1 Mutations in familial hyperaldosteronism affect glomerulosa cell function. **a** Under resting conditions, cells are hyperpolarized. **b** Glomerulosa cells respond to angiotensin II and hyperkalemia with depolarization, opening of voltage-gated calcium channels, increased expression of aldosterone synthase, and elevated aldosterone production. **c**–**f** Mutations in familial hyperaldosteronism either affect *CYP11B1* expression in FH-I, lead to depolarization (*CLCN2* mutations in FH-II, *KCNJ5* mutations in FH-III) or directly cause increased calcium permeability (*CACNA1H* in FH-IV, *CACNA1D* in PASNA syndrome)

and autonomous of its chronic regulator, renin/angiotensin II, levels of which are suppressed. Excessive aldosterone production leads to sodium retention and thus hypertension, and concurrently, excessive excretion of potassium, which, if severe and prolonged enough, may result in hypokalemia [12, 13].

In the lead up to the recent advances in unraveling its genetic bases, PA was already an area of major clinical and research interest, driven primarily by two lines of observation that arose in the 1990s and gathered further steam in the 2000s. The first of these was that PA is much more common than was previously suspected, accounting for possibly 5–13% of hypertensives (rather than the < 1% previously taught in textbooks of medicine) and at least 20% of patients with resistant hypertension [14•, 15–18]. Such a paradigm shift mainly occurred as a result of the availability of the plasma aldosterone/renin ratio (ARR) as a new method of screening for PA that was more sensitive than plasma potassium measurement [19, 20], and its application to a much wider population of hypertensive patients to include those with normal plasma potassium levels and not just those who were hypokalemic (now known to make up only a minority of patients with PA). The second major line of observation was that aldosterone excess has important adverse effects on the cardiovascular system and kidneys that are at least partly independent of its effects on blood pressure (BP), resulting in greater cardiovascular and renal morbidity compared with essential hypertensives matched for degree of hypertension [21–23]. The fact that this excess in morbidity is mitigated by the institution of specific surgical (unilateral adrenalectomy for patients with unilateral adrenal forms of PA) or medical (with pharmacological agents that antagonize aldosterone action) treatments but not by non-specific antihypertensives has emphasized the importance of detection of this disorder among the hypertensive population [13, 24, 25].

Familial Forms of PA

Familial Hyperaldosteronism Type I

In the first and so far most commonly identified familial form of PA, aldosterone production resulting from expression of the *CYP11B2*-derived coding sequences within the underlying hybrid *CYP11B1*/*CYP11B2* mutation is not only excessive, but regulated by adrenocorticotropin (ACTH) rather than angiotensin II, by virtue of the ACTH-responsive promoter elements within its *CYP11B1*-derived regulatory sequences. The hyperaldosteronism is therefore suppressible and the associated hypertension (which is often severe and can lead to early death from hypertensive stroke) readily controlled by the administration of glucocorticoids such as dexamethasone given in small doses that do not cause Cushingoid side effects [1, 2••, 3]. Plasma aldosterone levels are unresponsive to upright posture and to intravenous infusion of angiotensin II presumably because *CYP11B1* (unlike *CYP11B2*), and therefore, the hybrid gene lacks promotor elements that are angiotensin II-regulated [26]. Unlike wild-type *CYP11B2*, expression of which is confined to zona glomerulosa (ZG), the hybrid gene is expressed in zona fasciculata (ZF), again presumably by virtue of its *CYP11B1* (which is also expressed in ZF) regulatory elements. There, cortisol is available as a substrate for its enzyme activity which converts it into the so-called hybrid steroids, 18-hydroxy- and 18-oxo-cortisol, levels of which are elevated in this form of familial PA.

Diagnosis of this condition has been greatly facilitated by the development of rapid genetic methods of detecting the hybrid gene mutation in peripheral blood DNA [26]. This glucocorticoid-remediable form of aldosteronism was labeled familial hyperaldosteronism type I (FH-I) by Gordon and colleagues to distinguish it from familial PA which was non-glucocorticoid suppressible and which they labeled FH-II (see below) [27••].

Familial Hyperaldosteronism Type II—the Beginning

In the early 1990s, Gordon and coworkers began to report families with PA that was not relieved by glucocorticoid administration and not associated with the hybrid gene mutation causing FH-I [27••, 28••]. Unlike FH-I, some patients with FH-II had unilateral PA caused by aldosterone-producing adenomas (APAs) and were surgically curable by unilateral adrenalectomy. As the number of these families accumulated, it soon became evident that they far outnumbered those with FH-I, at least in Gordon's group's experience. Furthermore, FH-II appeared to be associated with a very diverse phenotype with clinical, biochemical, and morphological features that mimicked those of the much larger group of patients with apparently non-familial PA (ANFP). Hence, like ANFP, age of onset could vary from early to late adulthood, there was a roughly equal gender distribution, around a quarter of patients were hypokalemic, around 30% demonstrated unilateral (and the remainder bilateral) aldosterone overproduction on adrenal venous sampling, and at least 50% retained normal responsiveness of aldosterone to upright posture or angiotensin II infusion (with the remainder being unresponsive). Among the more than 40 families in this cohort, most have shown a pattern of transmission consistent with autosomal dominant inheritance, while others have too few affected members to tell [29]. Retrospectively, given the high prevalence of PA in the general population, many of the families with only two affected individuals likely represent chance familial associations of sporadic APAs and/or sporadic bilateral adrenal hyperplasia [30, 31].

Attempts to define the genetic basis (or bases) for FH-II at first were mainly directed at sequencing of selected candidate genes (including *CYP11B2* and *ATR1*). With these excluded, efforts moved to linkage analyses which at one point, using now outdated and relatively low resolution microsatellite markers, appeared to implicate a locus at chromosome 7p22. However, intense scrutiny of this locus, even with next-

generation sequencing techniques, failed to identify causative mutations [32–37].

Familial Hyperaldosteronism Type III

While the search for genetic causes of FH-II was ongoing, Geller and coworkers described a family with a new form of PA characterized by very severe, early (childhood) onset hyperaldosteronism, massively elevated hybrid steroid levels, marked diffuse hyperplasia of ZF bilaterally and lack of suppression of aldosterone with dexamethasone administration. Spironolactone failed to correct either the hypertension or hypokalemia, which only came under control following bilateral adrenalectomy [38]. The genetic basis for this condition, which Mulatero labeled FH-III [39], was a gain of function mutation in *KCNJ5*, which encodes an inwardly rectifying potassium channel and is expressed in adrenal cortex [4••]. Since then other families with mutations in varying positions within the exons of *KCNJ5* have been reported [5, 6, 30]. While most pedigrees so far described have demonstrated an early onset of hypertension, the severity of PA and hypertension has shown considerable diversity (ranging from severe and requiring bilateral adrenalectomy for control to mild and controllable with MR antagonists) as has the degree of abnormality on adrenal imaging, and is to a considerable degree dependent on the exact *KCNJ5* mutation inherited. Functionally, in vitro studies have shown that these mutations reduce the selectivity of the channel to potassium, permitting entry of sodium into the adrenal cortical cell, which results in cell depolarization and an influx of calcium into the cytoplasm, which in turns leads to increased expression of *CYP11B2* and aldosterone synthesis [4••, 5, 40].

Familial Hyperaldosteronism Type IV

Scholl et al. identified a gain-of-function germline mutation in *CACNA1H* in 5 of 40 subjects with onset of hypertension and PA at or before 10 years of age. This gene encodes a T-type voltage-gated calcium channel that has been implicated in the generation of voltage and calcium oscillations in ZG [41]. Of five relatives also found to have the mutation, three had early onset hypertension and two were normotensive as adults. The adrenal glands of mutation carriers appeared normal on imaging. Studies performed in vitro revealed that the mutation was associated with a shift of activation to a more hyperpolarized potential and slowed inactivation, putatively resulting in increased calcium influx and aldosterone production [7••, 8, 42].

Primary Aldosteronism with Seizures and Neurologic Abnormalities (PASNA)

Scholl and coworkers also identified germline mutations in *CACNA1D*, which encodes a voltage-gated L-type calcium channel highly expressed in ZG, in two of 100 individuals with early onset PA. Both mutations occurred de novo, and both subjects, in addition to PA, suffered seizures and a variety of neurological abnormalities. In vitro, both mutations caused channel activation at less depolarized membrane potentials, while one was also associated with nearly abolished channel inactivation. As with *CACNA1H* mutations, it is likely that these *CACNA1D* mutations directly cause increased calcium influx, resulting in increased aldosterone production [9••].

Somatic Mutations Causing PA

While germline mutations causing PA are rare, somatic mutations confined to aldosterone-producing adrenal lesions are much more common. At least 60% of APAs have so far been found to harbor somatic mutations, with *KCNJ5* the most commonly represented gene (mutated in approximately 40% of APAs and an even higher proportion in Asian cohorts [4••, 43, 44]), mutations in other ion channels and pumps (including *CACNA1D* [9••, 45], *ATP1A1* [45, 46•], and *ATP2B3* [46•]) being much less frequent [47]. By contrast, and for reasons that remain unclear, aldosterone-producing cell clusters (which are found in higher numbers than normal among patients with bilateral forms of PA) are most commonly associated with somatic mutations in *CACNA1D* [48, 49].

Elucidation of the Genetic Basis for PA in a Large Australian Family with FH-II

Among the original kindreds with FH-II described by Gordon and coworkers in the early 1990s was an Australian family, at first reported to have two affected members (a mother and daughter, then 46 and 25 years old, respectively) [27••]. Over time, the number of members found to have confirmed or suspected PA gradually increased, so that by 2018 [11••], nine had been identified with either borderline ($n = 1$) or frankly ($n = 8$) elevated ARR, and of these, six tested positive and one negative by fludrocortisone suppression testing (FST, used to definitively confirm or exclude PA) with the diagnosis of PA eventually spanning five generations. When plasma aldosterone levels at 0700 h or 0800 h following overnight recumbency were compared to those at 1000 h following 2 or 3 h sitting, standing, or walking, five showed lack of normal responsiveness to upright posture (defined as a rise of at least 50% over basal) while one was responsive.

Whole-exome sequencing using DNA extracted from three of the most floridly affected members, performed by Scholl et al. [11••], revealed a shared rare, damaging heterozygous variant in *CLCN2* (chr.3: g.184075850C>T, p.Arg172Gln), which encodes a chloride channel (ClC-2). The amino acid position was highly conserved among species from

invertebrates to humans. Good segregation with phenotype was seen among the pedigree, with the variant detected in five of the six subjects with positive FST, another two (who declined FST) with normal BP in adolescence but frankly elevated ARR and another with normal BP but borderline elevated ARR. Only one variant-positive subject was normotensive and had repeatedly normal ARR (consistent with incomplete penetrance). The single individual with hypertension raised ARR and positive FST but negative for the variant was the oldest affected member of the family whose PA was presumably sporadic. Interestingly, while plasma aldosterone levels in this individual showed normal responsiveness to upright posture, they were unresponsive in all five variant-positive subjects who underwent posture-stimulation testing. Furthermore, onset of hypertension was relatively late in this individual at 36 years old compared with that in hypertensive variant carriers (16–24 years old). Another hypertensive subject with raised ARR but normal FST who was also negative for the variant similarly showed normal responsiveness of aldosterone to upright posture and did not develop hypertension until age 39 years.

Other phenotypic features among the eight carriers of the *CLCN2* variant included (1) variable hypokalemia (present in three of them); (2) either normal morphology ($n = 1$), mild bulkiness ($n = 2$) or a small unilateral nodule ($n = 1$) on adrenal computed tomography (CT) in the five who underwent scanning; (3) bilateral adrenal production of aldosterone (as would be expected if PA was due to an inherited, germline mutation) in all three with confirmed PA who underwent adrenal venous sampling with both adrenal veins successfully cannulated; and (4) hypertension and hypokalemia (when present) readily corrected in response to treatment with either spironolactone or amiloride.

Prevalence of *CLCN2* Mutations Among Families with Early Onset PA

Analysis of *CLCN2* in 35 unrelated subjects diagnosed with PA by age 10 years and an additional 45 with PA by 20 years (all without mutations in other known PA disease genes) identified three further occurrences of p.Arg172Gln plus one occurrence each of four new rare and potentially damaging heterozygous variants (p.Met22Lys, p.Tyr26Asn, p.Lys362del, and p.Ser865Arg) at amino acid positions which showed variable (from moderate to high) degrees of conservation among species. Hence, the prevalence of *CLCN2* variants among unrelated patients with unexplained early onset PA was relatively high at 8/81 (9.9%). No instances of p.Arg172Gln were found among 375 patients with PA of onset after 20 years of age [11••].

Concurrently with the report of Scholl and coworkers, Fernandes-Rosa et al. reported identification of a mutation in *CLCN2* (p.Gly24Asp) in a single patient (a 9 year old severely hypertensive, hypokalemic female with markedly elevated ARR) among 12 with onset of PA before 25 years of age whose germline DNA was subjected to whole-exome sequencing, giving a prevalence of 8.3% [10•]. This individual demonstrated no morphological abnormality on adrenal CT, and her hypertension came under good control with spironolactone treatment. Phenotypic features of individuals with *CLCN2* mutations causing FH-II are summarized in Table 1.

Wild-Type CLCN2—Tissue Expression and Functional Characteristics

CLCN2 is widely expressed, with ClC-2 found in many tissues, including brain, lung, kidney, and intestines [50]. Data retrieved by Fernandes-Rosa and colleagues from a transcriptome analysis revealed high levels of expression of *CLCN2* in human adrenal cortex. These investigators also demonstrated substantial expression of ClC-2 in whole mouse adrenal by western blotting [10•]. Using immunohistochemical techniques, Scholl and coworkers were able to localize intra-adrenal human tissue expression of ClC-2 primarily to ZG, further supporting a role for this channel in aldosterone biosynthesis [11••].

ClC-2 channels are homodimers, with each subunit containing one conduction pore. The two pores can be opened and closed either individually through a fast gating mechanism, or together through a common slow gating mechanism [51]. Wild-type ClC-2 channels are closed at depolarized voltages and activate slowly at voltages negative to the chloride reversal potential. Using fluorescence lifetime imaging, Scholl et al. determined an intracellular chloride concentration in mouse ZG of nearly 75 mM, which would result in a chloride reversal potential of − 8 mV. The resting membrane potential of ZG cells is reported to be much more negative (approx. − 80 mV) [41], which would result in a slow activation of ClC-2. The resulting increase of the cellular chloride permeability and the efflux of chloride ions would then lead to depolarization of ZG cells.

In immature neurons, predominant chloride influx via the Na^+-K^+-$2Cl^-$ cotransporter 1 (NKCC1) leads to high intracellular chloride concentrations and excitatory chloride efflux through GABAergic signaling [52]. Whether similar mechanisms account for the high intracellular chloride concentration in the adrenal gland remains to be determined; the gene encoding NKCC1 (*SLC12A2*) is expressed at moderate levels in the adrenal gland (GTEx Analysis Release V7).

Transfection of human adrenal cancer HAC15 cells with wild type *CLCN2* led to a slight depolarization compared with non-transfected cells in perforated patch recordings. In human adrenal cancer H295R cells, *CLCN2* transection was

Table 1 Reported clinical features of subjects with CLCN2 variants [10, 11]

Subject no.	CLCN2 variant	Sex	Age at HTN Dx (years)	Age at PA Dx (years)	SBP/DBP (mm Hg)	Lowest plasma K+ (mmol/L)	Plasma aldosterone (pmol/L)	Plasma renin (mU/L)	ARR (pmol/mU)	Aldosterone responsiveness to upright posture	Adrenal CT	Lateralization on AVS
Normal range					< 140/90 in adults	3.5–5.5	100–950	8–40	2–75			
1	p.Arg172Gln	F	24	24	120/80 (on 3 meds)	2.7	1115.2	2.5	446.1	Unresponsive	A bulky left adrenal gland	Bilateral
2	p.Arg172Gln	M	NA	36	116/77	4.0	679.6	10.1	67.3	NA	NA	NA
3	p.Arg172Gln	F	19	19	150/100 (on 1 med)	3.3	760.1	< 1	380.1	Unresponsive	A small nodule in the left adrenal	Bilateral
4	p.Arg172Gln	F	20	20	140/100	3.8	735.1	< 1	367.6	Unresponsive	Mild bulky left adrenal	No
5	p.Arg172Gln	M	NA	NA	120/80 (age 19)	4.0	685.2	12.6	54.4	NA	NA	NA
6	p.Arg172Gln	M	17	17	130/85	4.0	726.8	< 1	366.4	NA	NA	NA
7	p.Arg172Gln	F	NA	14	94/62	4.0	588.1	< 1	294.1	Unresponsive	Normal	NA
8	p.Arg172Gln	F	16	16	170/110	3.4	707.4	< 1.68	353.7		Normal	Bilateral
9	p.Arg172Gln	F	15	15	150/100	2.9	1328.8	< 8.4	> 158.2	NA	NA	NA
10	p.Arg172Gln	F	32	32	118/68 (on 2 meds)	3.0	1284.4	< 8.4	> 152.9	NA	Normal	No
11	p.Arg172Gln	M	NA	13	121/75 (age 13)	4.4	332.9	< 5	> 66.6	NA	NA	NA
12	p.Arg172Gln	F	11	11	160/120	3.0	721.2	2.5	288.5	NA	Normal	NA
13	p.Arg172Gln	M	7	7	170/140	2.6	263.5	1.8	131.8	NA	NA	NA
14	p.Met22Lys	F	1	1	117/71	4.1	471.6	< 4.2	> 112.3	NA	NA	NA
15	p.Tyr26Asn	F	6	6	280/188 (age 20)	NA	2774	< 25.2	> 110.1	NA	NA	NA
16	p.Ser865Arg	M	15	20	130/100	2.6	1026.4	1.7	513.2	NA	Normal	NA
17	p.Lys362del	M	0.2	0.2	150/90	4.0	1769.8	< 1.3	884.9	NA	Normal	NA
18	p.Gly24Asp	F	9	9	172/100	1.8	2406	0.9	481.2	NA	NA	ND

For comparison within the table, plasma aldosterone levels for subject nos. 1–18 were converted to pmol/L, and the conversion factor for this was 1 ng/dL = 27.74 pmol/L; plasma renin activity was converted to plasma renin concentration, and the conversion factor for this was 1 ng/dL/h = 8.4 mU/L. For ARR calculation, renin values < 2 mU/L were transformed to 2

HTN hypertension, *Dx* diagnosis, *PA* primary aldosteronism, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *ARR* aldosterone-to-renin ratio, *CT* computed tomography, *AVS* adrenal venous sampling, *med* medication, *NA* not applicable, *ND* not determined

associated with greater levels of expression of *CYP11B2*. Furthermore, when cells were transfected with non-functional *CLCN2*, expression of *CYP11B2* was no greater than that in non-transfected control cells. These observations suggest that *CLCN2* plays a role in physiological aldosterone synthesis by promoting ZG cell depolarization, presumably resulting in activation of voltage-gated calcium channels and, in turn, upregulation of genes (and ultimately *CYP11B2*) involved in aldosterone production [10•, 11].

Functional Effects of CLCN2 Mutations

Expressing each of the five mutations identified among their subjects with early onset PA in HEK293T cells, Scholl and colleagues demonstrated all mutations to shift the ClC-2 activation curve to more positive voltages, increasing the open probability at the resting membrane potential. All mutations identified so far are in cytosolic domains or near the cytosolic ends of transmembrane helices of ClC-2 (Fig. 2a).

The p.Ser865Arg mutation within the carboxy-terminus of the protein was associated with slowed deactivation of both the individual (fast) and common gates and increased open probability of the fast gate, in alignment with earlier reports of single amino acid exchanges modifying channel gating [53]. The four other mutations accelerated activation and increased open probability of the common gate. The predicted effect of all identified changes was to enhance chloride efflux at physiological glomerulosa membrane potentials. Indeed, HAC15 cells transfected with p.Arg172Gln *CLCN2* showed larger constitutive depolarization (that is, a less negative resting membrane potential) compared to wild type transfected controls.

The p.Arg172Gln mutation (yellow in Fig. 2b) is located at the cytosolic end of the D-helix (purple in Fig. 2b). A serine residue at the opposite end of the D helix (red in Fig. 2b) is oriented towards the ion permeation pathway and may play a role in anion selectivity and channel gating. Interaction of the cytosolic end of the D-helix (including Arg172) with the carboxy-terminus could thus indirectly affect gating [54].

In H295R cells, transfection with each of the five mutated forms of *CLCN2* led to a greater increase in *CYP11B2* expression than with wild type *CLCN2*. Taken together, these findings would suggest that *CLCN2* mutations predispose ZG cells to depolarization (as with *KCNJ5* mutations causing FH-III), thus presumably resulting in an influx of calcium into the cytoplasm and ultimately increased expression of *CYP11B2* (as demonstrated in vitro) and therefore increased aldosterone production [11••].

When expressed in *Xenopus laevis* oocytes, Fernandes-Rosa and colleagues found the p.Gly24Asp mutant to be associated with marked activation of the ClC-2 channel, resulting in increased current amplitudes at potentials of −80 mV (the resting membrane potential of ZG cells). In H295R cells, both basal and angiotensin II-stimulated or potassium-stimulated *CYP11B2* expression and aldosterone production were increased in cells stably expressing the p.Gly24Asp channel compared to wild-type ClC-2. Similar to the mutations p.Met22Lys and p.Tyr26Asn identified by Scholl et al., p.Gly24Asp is located in a particular region of the cytosolic amino-terminus required for proper closing of ClC-2 (Fig. 2a). Deletion of this "inactivation domain" (amino acids 16–61) is known to lead to constitutive opening of the channel when recorded in *Xenopus laevis* oocytes [55] or perforated patch recordings from HEK293T cells [56], and similar observations have been made for the loop between helices J and K where Lys362 is located [10•, 57]. These observations again support the notion that *CLCN2* mutations associated with FH-II cause ZG cell depolarization, thereby opening voltage gated calcium channels and activating *CYP11B2* expression (and thus aldosterone production) via increased cytosolic calcium. Enhanced basal expression of *STAR* and *CYP21A2* was observed as well, suggesting that increased production of precursor steroids may also contribute to the increase in aldosterone synthesis [10•]. Future animal models of FH-II are expected to provide additional insight.

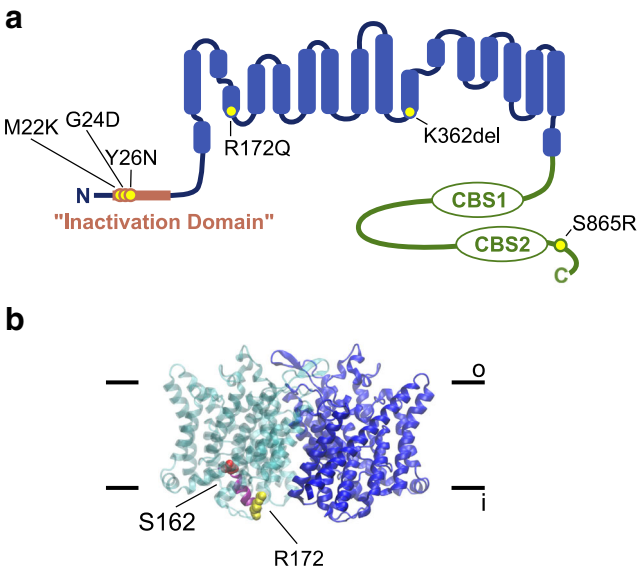

Fig. 2 Structure of ClC-2 and residues affected by mutations in FH-II. **a** Plotting the position of all identified CLCN2 mutations in FH-II onto a transmembrane topology model demonstrates their localization towards the cytosolic side (bottom) of the protein. Mutations Met22Lys, Gly24Asp, and Tyr26Asn cluster in the beginning of the proposed "inactivation domain" within the amino-terminus of the protein. Arg172Gln and K362del are positioned at the cytosolic end of the D- and K-helix, respectively. Mutation Ser865Arg is located in the cytosolic carboxy-terminus following the two structured cystathionine-beta-synthase (CBS) domains. **b** A view of the 3D structure of the transmembrane domains of the human ClC-1 highlights the position of Arg199 (yellow; homologous to Arg172 in ClC-2) at the cytosolic (i) side within one half of the protein. On the opposite side of the D-helix (purple) is Ser189 (red; homologous to Ser162 in ClC-2), which is known to be a part of the selectivity and gating mechanism in CLC proteins

Other Anion Channels in the Adrenal Zona Glomerulosa

Prior to the discovery of *CLCN2* mutations as a cause of FH-II, few studies had addressed a role of anion channels in ZG function and aldosterone production. These included the description in a review of a slowly activating very small chloride current at strongly negative voltages in rat glomerulosa cells (without available primary data and of unknown molecular identity) [58] and a Ras-dependent chloride current activated by ACTH [59], similarly described in rat ZG. Lastly, a study only published in abstract form [60] has described high and selective expression of *ANO4*, encoding a member of the anoctamine family of calcium-activated chloride channels, in the ZG. However, the roles of these channels in glomerulosa physiology and pathophysiology remain to be determined. Similarly, the physiological role of ClC-2 in ZG requires further characterization; potential aspects may include hyperpolarization-induced depolarization or the regulation of aldosterone production in response to changes in tonicity [50, 55].

Conclusions and Implications

The last decade has witnessed an explosion in knowledge regarding the genetics of PA, including its familial forms. Unlike previously reported genomic mutations, which have involved cation channels, the most recent addition to the list of genes mutated in familial and early onset PA is *CLCN2*, encoding an anion (chloride) channel. Subsequent analysis of both wild type and mutant forms has revealed, for the first time, a role for an anion channel in ZG membrane potential determination and normal aldosterone synthesis as well as in its overproduction and development of hypertension. Because mutations in *CLCN2* account for a substantial proportion of early onset PA, it would seem reasonable to undertake genetic testing in that situation in order to assist in establishing diagnosis both in the proband and in other family members. Confirmation of FH-II in this way may also facilitate selection of management approach, favoring treatment with medications (spironolactone, eplerenone, and amiloride) which block aldosterone action over unilateral or bilateral adrenalectomy, given the fact that patients with *CLCN2* mutations demonstrate bilateral production of aldosterone on AVS and good BP responses to medical treatment. The elucidation of the role of *CLCN2* in aldosterone production also opens up possibilities for new treatment approaches targeting this pathway specifically in patients with hypertensive disorders.

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Compliance with Ethical Standards

Conflict of Interest Heinrich Heine University Düsseldorf has filed a patent application: EP17209972, Diagnosis and Therapy of Primary Aldosteronism, with UIS as an inventor. Other authors declare no conflicts of interest relevant to this manuscript.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

1.• Sutherland DJ, Ruse JL, Laidlaw JC. Hypertension, increased aldosterone secretion and low plasma renin activity relieved by dexamethasone. Can Med Assoc J. 1966;95(22):1109–19 **This was the first description of familial PA (FH-I).**

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10.• Fernandes-Rosa FL, Daniil G, Orozco IJ, Goppner C, El Zein R, Jain V, et al. A gain-of-function mutation in the CLCN2 chloride channel gene causes primary aldosteronism. Nat Genet. 2018;50(3):355–61. https://doi.org/10.1038/s41588-018-0053-8 **This case report described a patient with early onset PA due to a CLCN2 mutation.**
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