Mini Review

HORMONE RESEARCH

Horm Res 2009;72:1–9
DOI: 10.1159/000224334

Received: March 20, 2009
Accepted: April 27, 2009
Published online: June 30, 2009

Clinical and Molecular Features of Type 1 Pseudohypoaldosteronism

Felix G. Riepe

Division of Paediatric Endocrinology, Department of Paediatrics, Christian Albrechts University, Kiel, Germany

Key Words
Pseudohypoaldosteronism · Salt loss · Aldosterone · Epithelial sodium channel · Mineralocorticoid receptor

Abstract
Pseudohypoaldosteronism (PHA) is a rare heterogeneous syndrome of mineralocorticoid resistance causing insufficient potassium and hydrogen secretion. PHA type 1 (PHA1) causes neonatal salt loss, failure to thrive, dehydration and circulatory shock. Two different forms of PHA1 can be distinguished on the clinical and genetic level, showing either a systemic or a renal form of mineralocorticoid resistance. This review provides an overview on transepithelial sodium reabsorption and on clinical features and the underlying molecular pathology of systemic and renal PHA1 caused by mutations in the subunit genes *(SCNN1A*, *SCNN1B*, *SCNN1G)* of the epithelial sodium channel (ENaC) and the mineralocorticoid receptor coding gene *NR3C2*. The in vitro investigation of several mutants has resulted in important progress in the understanding of the physiology of ENaC and the mineralocorticoid receptor. Some mutations are discussed in more detail to demonstrate some of these findings. A better clinical work-up of the patients suffering from PHA1 may delineate additional associations between the genotype and phenotype in the future.

Fluid balance, sodium and potassium homeostasis and blood pressure are regulated through the effect of aldosterone on polarized epithelial cells. The aldosterone signal is transduced by the mineralocorticoid receptor (MR) inducing the amiloride-sensitive epithelial sodium channel (ENaC) as the leading intracellular actor necessary for sodium conservation. Disruption of the intracellular MR signaling pathways leads to the clinical entity of pseudohypoaldosteronism (PHA). The delineation of the molecular alterations causing PHA contributed to the clarification of the intracellular factors responsible for salt homeostasis and their interplay as epithelial sodium transport machinery.

Mineralocorticoid Receptor

The MR acts as ligand-dependent transcription factor and is a member of the classic steroid-thyroid-retinoid nuclear receptor superfamily. MR is coded by the *NR3C2* gene. The *NR3C2* gene is localized on chromosome 4q31.1 and is composed of ten exons, whereas the first two (1α and 1β) are not translated [1]. Different tissue-specific mRNA isoforms are transcribed [2, 3] and the MR protein is further modified after translation by phosphorylation, ubiquitination, sumoylation and acetylation [4, 5]. These mechanisms are responsible for a tissue-specific modification of MR activity and specificity.

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0301–0163/09/0721–0001$26.00/0

Accessible online at:
www.karger.com/hre

Felix G. Riepe, MD
Division of Paediatric Endocrinology, Department of Paediatrics
Christian Albrechts University Kiel, University Hospital Schleswig-Holstein
Schwanenweg 20, DE–24105 Kiel (Germany)
Tel. +49 431 597 1622, Fax +49 431 597 1831, E-Mail friepe@pediatrics.uni-kiel.de

The mature MR protein consists of 984 amino acids and can be functionally subdivided in three domains, the N-terminal domain, the DNA-binding domain and the C-terminal ligand-binding domain (LBD). The N-terminal domain is highly conserved across different species. It contains two distinct activation function (AF) domains, referred to as AF1a and AF1b [4]. In addition, an inhibitory sequence was characterized. These regions are responsible for the recruitment of co-activators and co-repressors as well as a ligand-dependent interaction with the LBD [6]. The DNA-binding domain contains two zinc-fingers formed by two groups of four cysteines binding two zinc atoms. These zinc fingers establish the sequence-specific contact to the DNA [1]. Receptor dimerization is an additional modulator of target gene transcription. The crystal structure of the LBD has been studied recently [7, 8]. It consists of eleven α-helices and four antiparallel β-strands forming a helical structure with three different layers enclosing the ligand-binding pocket. The hydrophobic ligand-binding cavity is defined by polar sites composed of residues in helix 3, helix 5, and helix 11. The C-terminal helix 12 is attached to the remaining LBD in the ligand-bound conformation. A conserved LYFH motif follows this helix forming a hydrophobic core fixing helix 12 in the active conformation and hereby building a hydrophobic cleft on the surface of the protein serving as binding site for co-activators [9].

Epithelial Sodium Channel

ENaC constitutes the rate-limiting step in sodium reabsorption in the apical membrane of epithelia [10]. It is characterized by a high selectivity for sodium over potassium and a high affinity for the potassium-sparing diuretics amiloride and triamterene. ENaC is a heteromultimeric protein consisting of three subunits, termed α, β and γ ENaC [11]. The α, β and γ ENaC subunits are coded by the *SCNN1A* gene on chromosome 12p13, and the *SCNN1B* and the *SCNN1G* genes on chromosome 16p12. As deduced from the crystal structure of the ENaC orthologue ASIC1 channel, ENaC is likely a trimer consisting of three homologous subunits α, β, and γ [12]. However, good evidence alternatively supports the presence of two α ENaC subunits in the functional channel [13].

All three subunits share about 35% homology at the amino acid level and adopt the same topology, with two transmembrane α helices, a short intracellular amino- and carboxy-terminal end and a large extracellular loop corresponding to about two thirds of the protein. Numerous lysine residues in the amino-terminal region can be ubiquitinated and are key elements determining the half-life of the channel [14]. The extracellular loop contains several glycosylation sites as well as two cysteine-rich boxes (CRB1 and CRB2). CRB1 and CRB2 are critical for channel trafficking to the cell membrane. The intracellular carboxy-terminus contains several functional domains involved in the regulation of the number of channels present at the cell surface. A PPPXY motif is present on all three ENaC subunits. Deletions or missense mutations of this motif are found in patients affected by the Liddle syndrome causing arterial hypertension, hypoaldosteronism and hypokalemia [15]. A proline-rich domain in the carboxy-terminus resembles a SH3 protein-protein interaction domain and is involved in the interaction with the cytoskeleton [16].

Aldosterone Action on Epithelial Cells

Filtrated sodium is reabsorbed from the glomerular filtrate and potassium is secreted through a tight epithelium in the kidney. Sodium crosses the apical membrane and enters the epithelial cell through the ion-selective ENaC (fig. 1). Sodium is actively exchanged against potassium at the basolateral membrane mediated by the Na,K-ATPase [17]. This generates a lumen-negative voltage that drives potassium facilitated through a selective potassium channel (ROMK) into the lumen. Both cations are transported against an electrochemical potential with large transtubular concentration gradients.

Aldosterone regulates the final urinary sodium and potassium concentration in the renal collecting duct via the induction, activation and translation of various intracytoplasmatic, nuclear and transmembranous proteins [18]. The unbound MR is predominantly found in the cytoplasm associated with various chaperone proteins [19]. Aldosterone binds to MR after passively crossing the epithelial membrane. A conformational change in the receptor and a dissociation of the chaperone complex is induced [20]. The ligand-bound receptor translocates into the nucleus and accumulates in intranuclear clusters (fig. 2) [21]. Ligand-bound MR binds as dimer to response elements in the promoter regions of aldosterone target genes and initiates hormone-mediated gene transcription and repression [22]. The sodium transport machinery is initially activated by the transcription of signaling factors. Transepithelial sodium transport is hereby enhanced without a numerical increase in transport proteins. One early induced signaling factor is serum- and glucocorticoid-in-

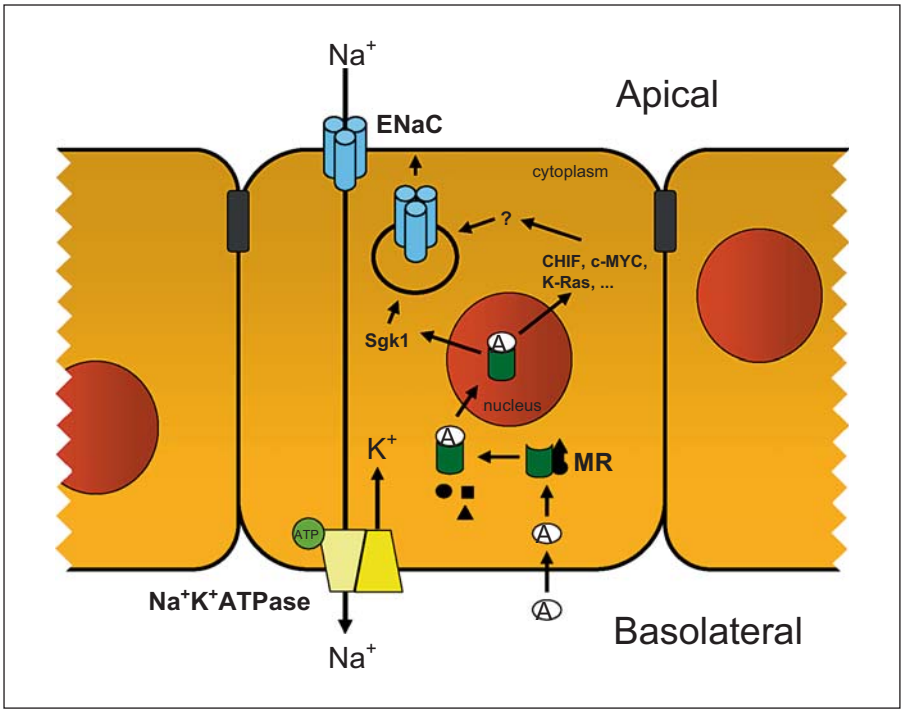

Fig. 1. Sketch of the sodium reabsorption system in epithelial cells. Aldosterone binds to the MR what initiates the dissociation of chaperone proteins. The steroid-receptor complex translocates into the nucleus, binds to MR-binding sites of the DNA and induces or represses the transcription of various genes. Among others, these genes include signaling factors like Sgk1 or the Ras family. By this, the epithelial sodium transport is increased without a numerical change of transport proteins by increasing the translocation of active ENaC and by increasing the open probability of ENaC. Later on, aldosterone activates the transcription and translation of additional ENaC subunits and Na^+,K^+-ATPase proteins [adapted from Expert Rev Endocrinol Metab 2007;2:407–419, with permission of Expert Reviews Ltd].

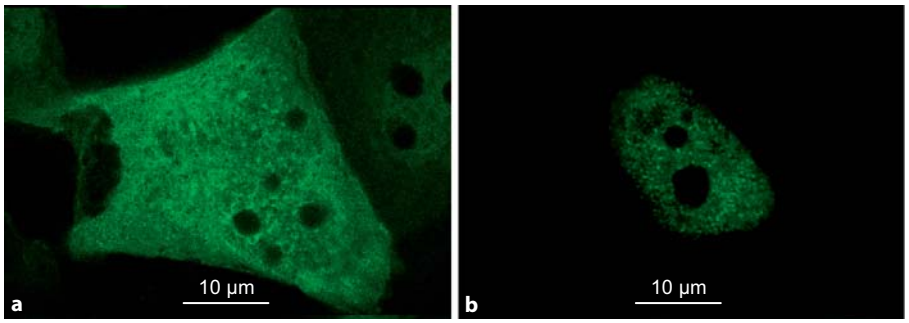

Fig. 2. Subcellular distribution of GFP-tagged MR in the absence of aldosterone (**a**) and 20 min after perfusion with 1 nM aldosterone (**b**). Rabbit cortical collecting duct cells were transiently transfected with a GFP-MR plasmid and grown on coverslips in steroid-free medium at 37°C. Confocal imaging revealed that the MR is located in the cytoplasm and the nucleus without aldosterone. After the perfusion with aldosterone, MR completely translocated intranuclear and formed typical clusters in response to the hormone.

duced kinase 1 (Sgk1) [23]. Activated Sgk1 phosphorylates the neuronal precursor cell expressed developmentally downregulated 2 (Nedd4-2) protein which allows binding of 14-3-3 proteins [24]. This facilitates the interaction of Nedd4-2 and ENaC, causing a reduced ubiquitination and hereby an accumulation of ENaC at the plasma membrane what enhances the transepithelial sodium transport [25]. ENaC-mediated sodium transport is further stimulated by a whole machinery of aldosterone-induced proteins [22]. In a later phase translation and allocation of ENaC proteins, basolateral Na,K^+-ATPase and apical K^+ channels (ROMK) are enhanced [26, 27].

Pseudohypoaldosteronism

PHA is a rare heterogeneous syndrome of mineralocorticoid resistance leading to insufficient potassium and hydrogen secretion. The common clinical features are hyperkalemia, metabolic acidosis and elevated plasma aldosterone levels. PHA has been classified into three distinct clinical forms (table 1) [28]. This classification includes primarily salt-losing syndromes, such as PHA type 1 (PHA1) and PHA type 3 (PHA3) and the potassium-retaining PHA type 2 (PHA2). All forms are caused by a mineralocorticoid resistance due to disturbances in

Table 1. Classification of pseudohypoaldosteronism (PHA)

PHA type		Inheritance	Phenotype	Affected genes
1	Systemic PHA	Recessive	Systemic sodium loss Hyponatremia, hyperkalemia, metabolic acidosis, elevated renin and aldosterone Pulmonary manifestation possible Subclinical phenotype in heterozygotes? Life-long disease	*SCNN1A*, *SCNN1B*, *SCNN1G*
	Renal PHA	Dominant	Renal sodium loss Hyponatremia, hyperkalemia, metabolic acidosis, elevated renin and aldosterone Amelioration of salt loss over years	*NR3C2*
2	Gordon syndrome	Dominant	Hyperkalemia, hypertension, hyperchloremic acidosis, suppressed renin, normal aldosterone	*WNK1*, *WNK4*
3	Secondary PHA	None	Secondary to sodium loss, e.g. with nephropathies or diarrhea Hyponatremia, hyperkalemia, metabolic acidosis, elevated renin and aldosterone, low glomerular filtration rate with nephropathies	none

the mineralocorticoid signal transduction. The clinical features and the underlying pathophysiology of PHA1 are described in detail in the following review. PHA2 is characterized by hyperkalemia and hypertension. It has been described by Gordon et al. [29] as heterogeneous syndrome with highly variable plasma aldosterone concentrations, suppressed plasma renin activity, various degrees of hyperchloremia and metabolic acidosis. Renal and adrenal functions are normal. PHA2 shows an autosomal dominant mode of inheritance. Deletions and mutations of two members of the WNK serine-threonine kinase family (WNK1 and WNK4) have been identified as disease-causing [30]. For detailed information the reader is referred to recent reviews on PHA2 [31–33]. Gordon syndrome can be treated with thiazide diuretics [34]. PHA3 comprises transient and secondary forms of salt-losing states caused by various pathologies of the kidney, intestinal tract or sweat glands. Nephropathies such as urinary tract infections and obstructive uropathies are the most frequent cause [35–38]. Contrary to PHA1 and PHA2, the glomerular filtration rate is decreased in these cases. The mechanism resulting in transient mineralocorticoid resistance is not clear.

Pseudohypoaldosteronism Type 1

PHA1 is characterized by neonatal salt loss resistant to mineralocorticoid treatment [39]. Laboratory findings are hyponatremia, hyperkalemia and metabolic acidosis. Plasma renin and aldosterone concentrations are highly elevated, reflecting a peripheral resistance of the kidney and other tissues to mineralocorticoids. The medical treatment of PHA1 consists of sodium supplementation. In addition, ion exchange resins may be necessary in order to lower elevated potassium levels. Two forms of PHA1 can be distinguished at the clinical and genetic level [40]. The severity of the disease and the phenotype of the two genetically different PHA1 forms vary noticeable.

Autosomal Dominant PHA1

Isolated renal resistance to aldosterone, leading to renal salt loss, hyponatremia, hyperkalemia, metabolic acidosis, failure to thrive, elevated plasma renin and aldosterone concentrations are the characteristics of autosomal dominant PHA1 (adPHA1) [40, 41]. The leading clinical sign is insufficient weight gain due to chronic dehydration. Hyperkalemia is generally mild and metabolic acidosis is not always detectable. The patients mainly manifest in early infancy. Medical treatment consists of sodium supplementation what is usually sufficient to lower the elevated potassium levels. Sodium supplementation becomes generally unnecessary by 1–3 years of age [39, 42], what is explained by the maturation of the renal salt conservation abilities by the replacement of distal sodium reabsorption through proximal parts of the tubulus. Overall adPHA1 is the milder PHA1 form as the salt loss is strictly restricted to the kidney. The clinical spectrum ranges from healthy unaffected patients, patients

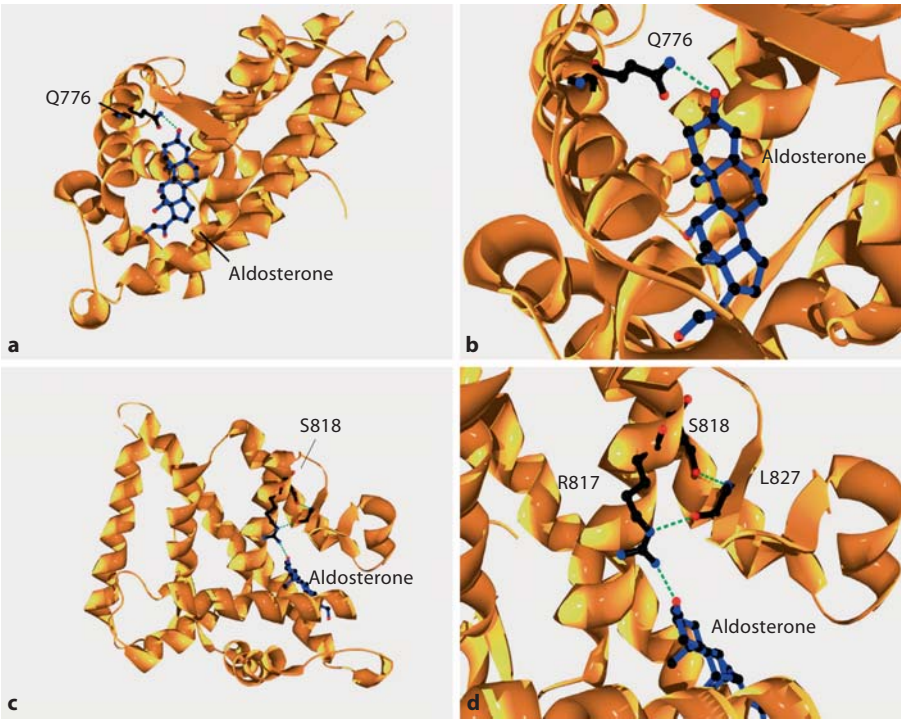

Fig. 3. Protein structure of MR (PDB code 2AA2). **a** Overview of the three-dimensional structure of MR depicting the residue Q776 in helix 3. Aldosterone is shown in blue. **b** Q776 is locking aldosterone in the ligand-binding pocket. **c** Overview of the three-dimensional structure of MR with the residue S818 in helix 5. Aldosterone is depicted in blue. **d** Close-up view of residues R817, S818 and L827. S818 is necessary to hold helix 5 and the β-sheet 1 in place. L827 in the β-sheet 1 stabilizes the residue R817 in the correct orientation. R817 is locking the A-ring of aldosterone in the ligand-binding pocket [adapted from Expert Rev Endocrinol Metab 2007; 2:407–419, with permission of Expert Reviews Ltd].

without electrolyte disturbances but elevated plasma renin and aldosterone levels to patients with clinically manifest renal salt loss [43]. Elevated aldosterone levels are the only biochemical marker of adPHA1 in adulthood [44]. However, reports from several families suggest that adult carriers of causative mutations might also have normal levels of aldosterone [45].

Molecular Cause of Autosomal Dominant PHA1

The underlying cause of adPHA1 is an inactivation of the human MR through mutations of the coding gene *NR3C2* [41]. A considerable amount of de novo mutations are found in *NR3C2* in affected individuals [43]. Familial *NR3C2* gene mutations can cause a very heterogeneous disease expression within one family. The reason for the incomplete penetrance of the phenotype is not known. More than 50 different mutations in the human *NR3C2* gene causing adPHA1 have been described. These mutations are spread throughout the gene. The first *NR3C2* mutations were identified in the late 1990s [41].

Nonsense mutations, frameshift mutations, splice site mutations and deletions of whole or parts of the gene lead to gross changes of the respective protein. All these sequence variations have been described in adPHA1. Nonsense mutations are found in all exons and lead to a more or less truncated MR protein. Most of the naturally occurring truncating mutations have not been studied in vitro. However, from artificial C-terminal deletion mutants of MR it is known that a deletion of the last four amino acids of the C-terminus already abolishes all ligand-binding abilities [46]. Intronic mutations of splice sites of the *NR3C2* gene as well as deletions of single *NR3C2* exons are occasionally detected in adPHA1 [41, 47].

Only two sequence variations (I180V and A241V) have been found in the N-terminal domain. The allele frequency of I180V and A241V in the general population varies between 5–17 and 0–97%, respectively, depending on the ethnic background [48]. These gene variations represent polymorphisms. No naturally occurring or artificial missense mutations within the AF-1 region have been detected to date. One may speculate that missense mutations in the N-terminal region have no major impact on the receptor function. The DNA-binding domain contains two zinc fingers and an α-helical substructure. Substitutions disturbing these domains, like C645S and R659S, have been reported in adPHA1 patients [47].

Mutations in the LBD most often disturb aldosterone binding. Several variants associated with adPHA1 have been described. By the use of three-dimensional protein

modeling the underlying structural disturbances have been delineated. This was especially useful for the functional characterization of different regions of the LBD. For example, by studying the inactivating mutation Q776R, helix 3 was detected as part of the hydrophobic ligand-binding pocket because Q776 locks the C3-ketone group of aldosterone (fig. 3a, b) [49]. The characterization of the variant S818L revealed the importance of β-sheet 1 for receptor activity [43]. S818 seems to be necessary for the stabilization of helix 5 and β-sheet 1 (fig. 3c, d). The position of β-sheet 1 is of elementary importance for keeping the residue R817 in correct orientation to lock the A-ring of aldosterone in the ligand-binding pocket. The investigation of other variants revealed several other functions of different parts of the LBD.

Genotype-Phenotype Associations

The clinical presentation of patients with adPHA1 manifesting in infancy includes hyponatremia due to renal salt loss in combination with elevated renin and hyperaldosteronism. Interestingly, no significant differences in clinical phenotypes attributable to the more than 50 different genotypes described so far have been detected. In vitro studies revealed that the underlying genetic variants have different residual transactivation abilities. However, this does not correlate with the severity of the clinical presentation. Furthermore, distinct differences of the disease severity between various affected members within one family or between unrelated individuals carrying the same mutation have been recollected consistent with a highly variable penetrance of the disease. This may be due to various confounding factors, e.g. infectious agents leading to enteritis or pyelonephritis, necessary to unmask an underlying adPHA1. In summary, a genotype-phenotype association cannot be drawn from the available data and each individual with assured *NR3C2* mutation, e.g. detected in family studies, has to be individually monitored in order to assess the sodium homeostasis.

Autosomal Recessive PHA1

The systemic arPHA1 follows an autosomal recessive trait of inheritance. The clinical manifestation is most often within the neonatal period with severe dehydration and hyponatremia due to systemic salt loss, including kidneys, colon and sweat and salivary glands. Elevated sodium concentration in sweat and absent nasal or rectal transepithelial voltage differences can be used as diagnostic tools. Hyponatremia and hyperkalemia are combined with elevated plasma renin and aldosterone concentrations reflecting end-organ resistance. Children suffering from arPHA1 often show pulmonary affections due to reduced sodium-dependent liquid absorption [50]. On clinical grounds, cough, tachypnea, fever and wheezing can be recollected [50–53]. Interestingly, no neonatal respiratory distress syndromes are reported in arPHA1 patients. In addition, phenotypes showing cholelithiasis, skin rashes mimicking milia rubra or dermal infections, salt loss via the meibomian glands or polyhydramnios are reported [28, 54–56]. The arPHA1 is a life-long disease showing no improvement over time [57]. Patients are prone to life-threatening salt-losing crises combined with severe hyperkalemia and dehydration. Medical management consists of extensive salt supplementation. Additional measures include the use of potassium-lowering agents like ion exchangers.

Molecular Cause of Autosomal Recessive PHA1

The systemic form of PHA1 is caused by inactivating mutations of the ENaC subunit genes *SCNN1A* (chromosome 12p13.31), *SCNN1B* (chromosome 16p12.1) and *SCNN1G* (chromosome 16p12.1). Various *SCNN1A*, *SCNN1B* and *SCNN1G* mutations are reported to date. The majority of mutations are frameshift or nonsense mutations leading to truncated or completely abnormal proteins, which are non-functional.

The first missense mutations were reported by Chang et al. [58] in five consanguineous kindreds originating from the Near East. Missense mutations in the different subunit genes allowed for gathering more information on the function and structure of ENaC. In a case with a mild clinical manifestation of arPHA1, the missense mutation G37S has been detected in the β ENaC subunit. The mutation is located in a conserved motif in the N-terminus upstream of the first transmembrane helix. Functional characterization of the residue in a *Xenopus laevis* oocyte expression system revealed a decrease in ENaC activity of 50% [59]. The introduction of homologous mutations into the α or γ subunits also reduced the sodium channel currents. The number of channel molecules present at the cell surface as well as single-channel conductance and ion selectivity were identical to that of wild type. However, single recordings of channels containing the mutants revealed a severe reduction in the open probability of the

channel. Thus, by studying the G37S mutation, a domain in the cytoplasmatic N-terminus of ENaC that controls channel gating has been identified.

The missense mutation S562L in the α ENaC subunit causing PHA-1 was found to be located at the start of the second transmembrane domain [53]. The serine residue at position 562 is lining the channel pore at its narrowest part. Together with the corresponding residues in the β and γ ENaC subunits, S562 forms a ENaC selectivity filter, allowing small ions like Na or Li to pass while excluding larger ions [60, 61]. Artificial mutations of residue 562 changing serine to amino acids of similar sizes make ENaC permeable for larger ions like K or Cs. On the contrary, artificial substitutions of serine 562 with aromatic residues inactivate the channel. Another naturally occurring sequence variation associated with arPHA1 has been detected at residue 562. The mutation S562P was found in a Somalian trait with the typical systemic PHA1 phenotype, however not showing any pulmonary manifestation. In vitro expression revealed a dramatic loss of function of the S562P mutant. The ENaC channel complex is correctly arranged but almost non-conducting likely due to structural changes in the critical region of the selectivity filter. Co-expression of the mutant S562P α ENaC subunit with wild-type α ENaC in different ratios showed that the S562P α ENaC mutation exerts a dominant negative effect on the function of the heteromeric ENaC channel. As the expression of the mutant S562P is comparable to ENaC wild type and as it retains its ability to assemble with β and γ ENaC subunits, the dominant negative effect is consistent with two α-subunits in the ENaC channel, where the S562P mutation in either one of the two subunits alters the channel function. It is not clear yet how such a two α-subunit stoichiometry of ENaC can be reconciled with the trimeric nature of the channel as suggested by the ASIC crystal structure [12]. All heterozygote carriers in the family reflecting in vivo compound heterozygosity of the mutant and wild-type allele were clinically free of symptoms. No episodes of salt loss and no increased rates of pulmonary infections were recollected. Plasma renin activity, aldosterone and blood pressure were within the normal limits for age. However, elevated sodium and chloride levels have been repeatedly recorded in sweat tests of the heterozygous parents. It may be possible that elevated sweat sodium and chloride levels in carriers of *SCNN1A* mutations reflect a subclinical phenotype caused by the dominant negative effect of a mutation on ENaC activity. A comparable finding was made with heterozygous mutations of α ENaC in mice, which produces modest reduction in extracellular volume, blood pressure, potassium excretion and sodium conservation in combination with a very low sodium diet [62].

Genotype-Phenotype Associations

Although the clinical syndrome of arPHA1 has been well known since the 1950s, few reports describe clinical characteristics in detail. The residual function of ENaC when tested in a heterologous system such as the *Xenopus* system may give some hint on the clinical severity. One patient with a partially inactivating G327C mutation showed significantly less salt-losing crises and had a normalization of the initially elevated plasma renin activity and aldosterone levels over time [63]. On the contrary, patients with a completely inactivating mutation have no normalization of these parameters. However, it cannot be answered at this time whether the diversity of mutations correspond to different clinical phenotypes on a larger scale as sufficient clinical reports are missing.

Conclusion

In summary, PHA1 is a potentially life-threatening salt-losing disease in neonates and infants. The systemic form persists into adulthood, whereas the salt loss in the renal form ameliorates during childhood. PHA1 is a state of mineralocorticoid resistance caused by inactivating mutations in the ENaC subunits genes in the case of arPHA1 or by inactivating mutations in the MR-coding gene in the case of adPHA1. The in vitro investigation of these inactivating mutations provided novel insights into the physiology and three-dimensional structure of ENaC and MR. However, due to the rarity of the disease, the clinical descriptions and investigations available do not allow to conclude whether there is a genotype-phenotype correlation beyond the categories of arPHA1 and adPHA1. Therefore, future studies focusing on the clinical work-up of the disease are warranted.

References

1 Arriza JL, Weinberger C, Cerelli G, Glaser TM, Handelin BL, Housman DE, Evans RM: Cloning of human mineralocorticoid receptor complementary DNA: structural and functional kinship with the glucocorticoid receptor. Science 1987;237:268–275.

2 Zennaro MC, Farman N, Bonvalet JP, Lombes M: Tissue-specific expression of α and β messenger ribonucleic acid isoforms of the human mineralocorticoid receptor in normal and pathological states. J Clin Endocrinol Metab 1997;82:1345–1352.

3 Zennaro MC, Le Menuet D, Lombes M: Characterization of the human mineralocorticoid receptor gene 5′-regulatory region: evidence for differential hormonal regulation of two alternative promoters via nonclassical mechanisms. Mol Endocrinol 1996; 10:1549–1560.

4 Pascual-Le Tallec L, Lombes M: The mineralocorticoid receptor: a journey exploring its diversity and specificity of action. Mol Endocrinol 2005;19:2211–2221.

5 Seeler JS, Dejean A: Nuclear and unclear functions of sumo. Nat Rev Mol Cell Biol 2003;4:690–699.

6 Rogerson FM, Fuller PJ: Interdomain interactions in the mineralocorticoid receptor. Mol Cell Endocrinol 2003;200:45–55.

7 Fagart J, Huyet J, Pinon GM, Rochel M, Mayer C, Rafestin-Oblin ME: Crystal structure of a mutant mineralocorticoid receptor responsible for hypertension. Nat Struct Mol Biol 2005;12:554–555.

8 Bledsoe RK, Madauss KP, Holt JA, Apolito CJ, Lambert MH, Pearce KH, Stanley TB, Stewart EL, Trump RP, Willson TM, Williams SP: A ligand-mediated hydrogen bond network required for the activation of the mineralocorticoid receptor. J Biol Chem 2005;280:31283–31293.

9 Li Y, Suino K, Daugherty J, Xu HE: Structural and biochemical mechanisms for the specificity of hormone binding and coactivator assembly by mineralocorticoid receptor. Mol Cell 2005;19:367–380.

10 Garty H, Palmer LG: Epithelial sodium channels: function, structure, and regulation. Physiol Rev 1997;77:359–396.

11 Canessa CM, Schild L, Buell G, Thorens B, Gautschi I, Horisberger JD, Rossier BC: Amiloride-sensitive epithelial Na^+ channel is made of three homologous subunits. Nature 1994;367:463–467.

12 Jasti J, Furukawa H, Gonzales EB, Gouaux E: Structure of acid-sensing ion channel 1 at 1.9 Å resolution and low pH. Nature 2007;449: 316–323.

13 Anantharam A, Palmer LG: Determination of epithelial Na^+ channel subunit stoichiometry from single-channel conductances. J Gen Physiol 2007;130:55–70.

14 Staub O, Gautschi I, Ishikawa T, Breitschopf K, Ciechanover A, Schild L, Rotin D: Regulation of stability and function of the epithelial Na^+ channel (ENaC) by ubiquitination. EMBO J 1997;16:6325–6336.

15 Shimkets RA, Warnock DG, Bositis CM, Nelson-Williams C, Hansson JH, Schambelan M, Gill JR Jr, Ulick S, Milora RV, Findling JW, et al: Liddle's syndrome: heritable human hypertension caused by mutations in the β-subunit of the epithelial sodium channel. Cell 1994;79:407–414. (*This study delineates activating mutations in the carboxy-terminal region of the ENaC β-subunit as cause for Liddle's syndrome, an autosomal dominant form of hypertension.)*

16 Rotin D, Bar-Sagi D, O'Brodovich H, Merilainen J, Lehto VP, Canessa CM, Rossier BC, Downey GP: An SH3 binding region in the epithelial Na^+ channel (α rENaC) mediates its localization at the apical membrane. EMBO J 1994;13:4440–4450.

17 Pearce D, Bhargava A, Cole TJ: Aldosterone: its receptor, target genes, and actions. Vitam Horm 2003;66:29–76.

18 Fuller PJ, Young MJ: Mechanisms of mineralocorticoid action. Hypertension 2005;46: 1227–1235.

19 Ratajczak T, Ward BK, Minchin RF: Immunophilin chaperones in steroid receptor signalling. Curr Top Med Chem 2003;3:1348–1357.

20 Hellal-Levy C, Fagart J, Souque A, Rafestin-Oblin ME: Mechanistic aspects of mineralocorticoid receptor activation. Kidney Int 2000;57:1250–1255.

21 Fejes-Toth G, Pearce D, Naray-Fejes-Toth A: Subcellular localization of mineralocorticoid receptors in living cells: effects of receptor agonists and antagonists. Proc Natl Acad Sci USA 1998;95:2973–2978.

22 Robert-Nicoud M, Flahaut M, Elalouf JM, Nicod M, Salinas M, Bens M, Doucet A, Wincker P, Artiguenave F, Horisberger JD, Vandewalle A, Rossier BC, Firsov D: Transcriptome of a mouse kidney cortical collecting duct cell line: effects of aldosterone and vasopressin. Proc Natl Acad Sci USA 2001; 98:2712–2716.

23 Pearce D: Sgk1 regulation of epithelial sodium transport. Cell Physiol Biochem 2003;13: 13–20.

24 Bhalla V, Daidie D, Li H, Pao AC, LaGrange LP, Wang J, Vandewalle A, Stockand JD, Staub O, Pearce D: Serum- and glucocorticoid-regulated kinase 1 regulates ubiquitin ligase neural precursor cell-expressed, developmentally down-regulated protein 4-2 by inducing interaction with 14-3-3. Mol Endocrinol 2005;19:3073–3084.

25 Snyder PM, Steines JC, Olson DR: Relative contribution of Nedd4 and Nedd4-2 to ENaC regulation in epithelia determined by RNA interference. J Biol Chem 2004;279:5042–5046.

26 Mick VE, Itani OA, Loftus RW, Husted RF, Schmidt TJ, Thomas CP: The α-subunit of the epithelial sodium channel is an aldosterone-induced transcript in mammalian collecting ducts, and this transcriptional response is mediated via distinct *cis*-elements in the 5′-flanking region of the gene. Mol Endocrinol 2001;15:575–588.

27 Yoo D, Kim BY, Campo C, Nance L, King A, Maouyo D, Welling PA: Cell surface expression of the ROMK (Kir-1.1) channel is regulated by the aldosterone-induced kinase, SGK-1, and protein kinase A. J Biol Chem 2003;278:23066–23075.

28 Kuhnle U: Pseudohypoaldosteronism: mutation found, problem solved? Mol Cell Endocrinol 1997;133:77–80.

29 Gordon RD: Syndrome of hypertension and hyperkalemia with normal glomerular filtration rate. Hypertension 1986;8:93–102.

30 Wilson FH, Disse-Nicodeme S, Choate KA, Ishikawa K, Nelson-Williams C, Desitter I, Gunel M, Milford DV, Lipkin GW, Achard JM, Feely MP, Dussol B, Berland Y, Unwin RJ, Mayan H, Simon DB, Farfel Z, Jeunemaitre X, Lifton RP: Human hypertension caused by mutations in WNK kinases. Science 2001;293:1107–1112.

31 Kahle KT, Wilson FH, Lifton RP: Regulation of diverse ion transport pathways by WNK4 kinase: a novel molecular switch. Trends Endocrinol Metab 2005;16:98–103.

32 Xie J, Craig L, Cobb MH, Huang CL: Role of with-no-lysine [K] kinases in the pathogenesis of Gordon's syndrome. Pediatr Nephrol 2006;21:1231–1236.

33 Subramanya AR, Yang CL, McCormick JA, Ellison DH: WNK kinases regulate sodium chloride and potassium transport by the aldosterone-sensitive distal nephron. Kidney Int 2006;70:630–634.

34 Disse-Nicodeme S, Achard JM, Desitter I, Houot AM, Fournier A, Corvol P, Jeunemaitre X: A new locus on chromosome 12p13.3 for pseudohypoaldosteronism type II, an autosomal dominant form of hypertension. Am J Hum Genet 2000;67:302–310.

35 Bulchmann G, Schuster T, Heger A, Kuhnle U, Joppich I, Schmidt H: Transient pseudohypoaldosteronism secondary to posterior urethral valves – a case report and review of the literature. Eur J Pediatr Surg 2001;11: 277–279.

36 Maruyama K, Watanabe H, Onigata K: Reversible secondary pseudohypoaldosteronism due to pyelonephritis. Pediatr Nephrol 2002;17:1069–1070.

37 Dolezel Z, Starha J, Novotna D, Dostalkova D: Secondary pseudohypoaldosteronism in an infant with pyelonephritis. Bratisl Lek Listy 2004;105:435–437.

38 Perez-Brayfield MR, Gatti J, Smith E, Kirsch AJ: Pseudohypoaldosteronism associated with ureterocele and upper pole moiety obstruction. Urology 2001;57:1178.

39 Cheek D, Perry J: A salt-wasting syndrome in infancy. Arch Dis Child 1958;33:252–256.
40 Geller DS: Mineralocorticoid resistance. Clin Endocrinol (Oxf) 2005;62:513–520.
41 Geller DS, Rodriguez-Soriano J, Vallo Boado A, Schifter S, Bayer M, Chang SS, Lifton RP: Mutations in the mineralocorticoid receptor gene cause autosomal dominant pseudohypoaldosteronism type 1. Nat Genet 1998;19:279–281. (*First report on NR3C2 mutations in adPHA1.)*
42 Kuhnle U, Nielsen MD, Tietze HU, Schroeter CH, Schlamp D, Bosson D, Knorr D, Armanini D: Pseudohypoaldosteronism in eight families: different forms of inheritance are evidence for various genetic defects. J Clin Endocrinol Metab 1990;70:638–641.
43 Riepe FG, Finkeldei J, de Sanctis L, Einaudi S, Testa A, Karges B, Peter M, Viemann M, Grotzinger J, Sippell WG, Fejes-Toth G, Krone N: Elucidating the underlying molecular pathogenesis of NR3C2 mutants causing autosomal dominant pseudohypoaldosteronism type 1. J Clin Endocrinol Metab 2006;91:4552–4561.
44 Geller DS, Zhang J, Zennaro MC, Vallo-Boado A, Rodriguez-Soriano J, Furu L, Haws R, Metzger D, Botelho B, Karaviti L, Haqq AM, Corey H, Janssens S, Corvol P, Lifton RP: Autosomal dominant pseudohypoaldosteronism type 1: mechanisms, evidence for neonatal lethality, and phenotypic expression in adults. J Am Soc Nephrol 2006;17:1429–1436.
45 Riepe FG, Krone N, Morlot M, Peter M, Sippell WG, Partsch CJ: Autosomal-dominant pseudohypoaldosteronism type 1 in a Turkish family is associated with a novel nonsense mutation in the human mineralocorticoid receptor gene. J Clin Endocrinol Metab 2004;89:2150–2152.
46 Couette B, Jalaguier S, Hellal-Levy C, Lupo B, Fagart J, Auzou G, Rafestin-Oblin ME: Folding requirements of the ligand-binding domain of the human mineralocorticoid receptor. Mol Endocrinol 1998;12:855–863.
47 Pujo L, Fagart J, Gary F, Papadimitriou DT, Claes A, Jeunemaitre X, Zennaro MC: Mineralocorticoid receptor mutations are the principal cause of renal type 1 pseudohypoaldosteronism. Hum Mutat 2007;28:33–40.
48 Balsamo A, Cicognani A, Gennari M, Sippell WG, Menabo S, Baronio F, Riepe FG: Functional characterization of naturally occurring *NR3C2* gene mutations in Italian patients suffering from pseudohypoaldosteronism type 1. Eur J Endocrinol 2007;156:249–256.
49 Sartorato P, Lapeyraque AL, Armanini D, Kuhnle U, Khaldi Y, Salomon R, Abadie V, Di Battista E, Naselli A, Racine A, Bosio M, Caprio M, Poulet-Young V, Chabrolle JP, Niaudet P, De Gennes C, Lecornec MH, Poisson E, Fusco AM, Loli P, Lombes M, Zennaro MC: Different inactivating mutations of the mineralocorticoid receptor in fourteen families affected by type 1 pseudohypoaldosteronism. J Clin Endocrinol Metab 2003;88:2508–2517.
50 Kerem E, Bistritzer T, Hanukoglu A, Hofmann T, Zhou Z, Bennett W, MacLaughlin E, Barker P, Nash M, Quittell L, Boucher R, Knowles MR: Pulmonary epithelial sodium-channel dysfunction and excess airway liquid in pseudohypoaldosteronism. N Engl J Med 1999;341:156–162.
51 Hanukoglu A, Bistritzer T, Rakover Y, Mandelberg A: Pseudohypoaldosteronism with increased sweat and saliva electrolyte values and frequent lower respiratory tract infections mimicking cystic fibrosis. J Pediatr 1994;125:752–755.
52 Marthinsen L, Kornfalt R, Aili M, Andersson D, Westgren U, Schaedel C: Recurrent pseudomonas bronchopneumonia and other symptoms as in cystic fibrosis in a child with type I pseudohypoaldosteronism. Acta Paediatr 1998;87:472–474.
53 Schaedel C, Marthinsen L, Kristoffersson AC, Kornfalt R, Nilsson KO, Orlenius B, Holmberg L: Lung symptoms in pseudohypoaldosteronism type 1 are associated with deficiency of the α-subunit of the epithelial sodium channel. J Pediatr 1999;135:739–745.
54 Hanaki K, Ohzeki T, Iitsuka T, Nagata I, Urashima H, Tsukuda T, Nagaishi J, Shiraki K, Shimizu N, Kaibara N: An infant with pseudohypoaldosteronism accompanied by cholelithiasis. Biol Neonate 1994;65:85–88.
55 Martin JM, Calduch L, Monteagudo C, Alonso V, Garcia L, Jorda E: Clinico-pathological analysis of the cutaneous lesions of a patient with type 1 pseudohypoaldosteronism. J Eur Acad Dermatol Venereol 2005;19:377–379.
56 Wong GP, Levine D: Congenital pseudohypoaldosteronism presenting in utero with acute polyhydramnios. J Matern Fetal Med 1998;7:76–78.
57 Zennaro MC, Lombes M: Mineralocorticoid resistance. Trends Endocrinol Metab 2004;15:264–270.
58 Chang SS, Grunder S, Hanukoglu A, Rosler A, Mathew PM, Hanukoglu I, Schild L, Lu Y, Shimkets RA, Nelson-Williams C, Rossier BC, Lifton RP: Mutations in subunits of the epithelial sodium channel cause salt wasting with hyperkalaemic acidosis, pseudohypoaldosteronism type 1. Nat Genet 1996;12:248–253. (*First report on SCNN1 mutations in arPHA1.)*
59 Grunder S, Firsov D, Chang SS, Jaeger NF, Gautschi I, Schild L, Lifton RP, Rossier BC: A mutation causing pseudohypoaldosteronism type 1 identifies a conserved glycine that is involved in the gating of the epithelial sodium channel. EMBO J 1997;16:899–907.
60 Kellenberger S, Gautschi I, Schild L: A single point mutation in the pore region of the epithelial Na^+ channel changes ion selectivity by modifying molecular sieving. Proc Natl Acad Sci USA 1999;96:4170–4175.
61 Kellenberger S, Hoffmann-Pochon N, Gautschi I, Schneeberger E, Schild L: On the molecular basis of ion permeation in the epithelial Na^+ channel. J Gen Physiol 1999;114:13–30.
62 Hummler E, Barker P, Gatzy J, Beermann F, Verdumo C, Schmidt A, Boucher R, Rossier BC: Early death due to defective neonatal lung liquid clearance in α-EnAC-deficient mice. Nat Genet 1996;12:325–328.
63 Hanukoglu A, Edelheit O, Shriki Y, Gizewska M, Dascal N, Hanukoglu I: Renin-aldosterone response, urinary Na/K ratio and growth in pseudohypoaldosteronism patients with mutations in epithelial sodium channel (EnAC) subunit genes. J Steroid Biochem Mol Biol 2008;111:268–274.