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Review

Syndromes of impaired ion handling in the distal nephron: pseudohypoaldosteronism and familial hyperkalemic hypertension

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ABSTRACT

The distal nephron, which is the site of the micro-regulation of water absorption and ion handling in the kidneys, is under the control of aldosterone. Impairment of the mineralocorticoid signal transduction pathway results in resistance to the action of aldosterone and of mineralocorticoids in general. Herein, we review two syndromes in which ion handling in the distal nephron is impaired: pseudohypoaldosteronism (PHA) and familial hyperkalemic hypertension (FHH). PHA is a rare inherited syndrome characterized by mineralocorticoid resistance, which leads to salt loss, hypotension, hyperkalemia and metabolic acidosis. There are two types of this syndrome: a renal (autosomal dominant) type due to mutations of the mineralocorticoid receptor (MR), and a systemic (autosomal recessive) type due to mutations of the epithelial sodium channel (ENaC). There is also a transient form of PHA, which may be due to urinary tract infections, obstructive uropathy or several medications. FHH is a rare autosomal dominant syndrome, characterized by salt retention, hypertension, hyperkalemia and metabolic acidosis. In FHH, mutations of WNK (with-no-lysine kinase) 4 and 1 alter the activity of several ion transportation systems in the distal nephron. The study of the pathophysiology of PHA and FHH greatly elucidated our understanding of the renin-angiotensin-aldosterone system function and ion handling in the distal nephron. The physiological role of the distal nephron and the pathophysiology of diseases in which the renal tubule is implicated may hence be better understood and, based on this understanding, new drugs can be developed.

Key words: Aldosterone, Distal nephron, Epithelial sodium channel, Familial hyperkalemic hypertension, Mineralocorticoid receptor, Pseudohypoaldosteronism, With-no-lysine kinase

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INTRODUCTION

The distal nephron is the site of the micro-regulation of water reabsorption and ions reabsorption or excretion in the kidneys.[1-3] It is divided into three segments: the distal convoluted tubule (DCT) (which

is further divided into DCT-1 (early DCT) and DCT-2 (late DCT)[4,5]), the connecting tubule (CNT) and the cortical collecting duct (CCD).[3] At the apical membrane of DCT, the Na^+-Cl^- cotransporter (NCC) reabsorbs sodium and chloride[6-8] (reabsorption of 7% of the filtered sodium).[2,9,10] At the apical membrane of DCT-2, CNT and CCD, sodium is reabsorbed through the epithelial sodium channel (ENaC)[6,7] (reabsorption of 2% of the filtered sodium[2,9,10]), reducing the luminal potential and therefore promoting potassium excretion through the renal outer medullary potassium channel (ROMK)[1,2,4,9-12] in CNT and CCD[13] and hydrogen excretion.[1,9-11] (ROMK is also expressed in the thick ascending limb of Henle's loop (TAL)).[13] ROMK is constitutively open and responsible for potassium excretion when luminal fluid flow is at the baseline.[14-17] The maxi-K (or BK) potassium channel at the apical membrane of the distal renal tubule is relatively quiescent at the baseline, but it excretes potassium when luminal fluid flow is increased.[15,16,18,19] In CNT and CCD, chloride is mostly reabsorbed through the paracellular pathway via claudins (paracellular ion channels of tight junctions).[12,18,20] Moreover, sodium and chloride reabsorption is followed by water, which is passively reabsorbed.[3,10,15,21] Among the hormones that regulate transportation systems of the distal nephron, aldosterone plays a major role[1,3,9,22] and induces its actions through its binding to the mineralocorticoid receptor (MR), its intracellular receptor.[1,9-11,15,23-28] Herein, we review two syndromes in which ion handling in the distal nephron is impaired: pseudohypoaldosteronism (PHA) and familial hyperkalemic hypertension (FHH).

PSEUDOHYPOALDOSTERONISM (PHA)

PHA is a rare heterogeneous inherited syndrome[1,9,23-25,29-31] caused by decreased response to aldosterone.[1,9,12,23,24,26,28,32] PHA begins during the neonatal or early infant period and is characterized by salt wasting, dehydration, hypotension, weakness, lethargy, anorexia, vomiting, insufficient weight gain and failure to thrive. Laboratory findings include hyponatremia, hyperkalemia, metabolic acidosis, increased sodium and decreased potassium excretion in urine, high plasma renin levels and high plasma and urine aldosterone levels,[1,9,11,22-31,33] reflecting the peripheral resistance of the kidneys and other tissues to mineralocoricoids,[1,9,23,26,29] while adrenal function and glomerular filtration rate (GFR) are normal.[1,22,26,29] Patients do not respond to administration of exogenous mineralocorticoids.[22,23,25,26,30] High plasma concentrations of renin and aldosterone combined with unresponsiveness to administration of exogenous mineralocorticoids can be used for diagnosing PHA. Elevated aldosterone plasma values may persist even after normalization of the clinical picture.[1,25] There are two types of PHA: renal (or autosomal dominant) and systemic (or autosomal recessive).[34,35]

RENAL PHA (AUTOSOMAL DOMINANT PHA)

Renal PHA is characterized by resistance to aldosterone that affects only the kidneys,[1,11,23-26,29] leading to renal salt loss.[1,9,23-26] Salt supplementation (3-20 mEq/kg/day of sodium[1]), which is usually sufficient to lower increased potassium levels, and potassium binding resins in more severe cases, are used to treat patients with renal PHA.[1,9,10,23-26,28,29] Prognosis is good and patients usually respond well to the treatment, which generally becomes unnecessary after early childhood,[1,9,10,23-26,28,29] usually between 1 and 3 years old.[23,35] Afterwards, symptoms subside and patients are able to maintain electrolyte homeostasis without salt supplementation.[1,9-11,23-26,28,29] Nevertheless, symptoms and biochemical abnormalities may reappear during periods of dietary salt restriction.[28] Renal PHA is milder than systemic PHA, in which patients require life-long treatment.[1,9,10,12,23-26,28,29] The salt loss is restricted to the kidneys[1,9,11,23-26,29] and increased sodium levels are present only in urine and not in stool, sweat or saliva, and there is no pulmonary involvement.[9,24,26] Additionally, hyperkalemia is usually mild and metabolic acidosis is not always detectable.[23] The clinical phenotype is also normal after childhood and increased renin and aldosterone levels are the only possible biochemical markers in adults.[36,37]

The underlying cause of renal PHA is loss-of-function mutations in the MR gene,[36-49] which is the *NR3C2* gene and is located on chromosome 4q31.1.[50,51] The mutations of the *NR3C2* gene are inherited in the autosomal dominant pattern,[36-49] but there are also some sporadic cases[36,38-40,42,44-46] which occur due to de novo mutations.[36,38,40,44-46] They have the same prognosis and present the same clinical and laboratory findings as the inherited cases.[24,25] More than 50

different mutations of the *NR3C2* gene have been found,[1,23] including nonsense, missense, frameshift (insertion or deletion) and splice site mutations in the coding regions and in the intron/exon splice junctions throughout the gene and deletions of whole or parts of the gene, which result in changes in the structure of MR and in a MR with impaired function.[36-49,52] Mutations of the *NR3C2* gene have been identified in most studied cases of renal PHA.[36-49] Nevertheless, there are several autosomal dominant and sporadic cases where no mutations of the *NR3C2* gene have been detected,[40,42,45,46,53-56] leading to the hypothesis that in the remaining cases, disease-causing mutations occur in non-coding regions of the *NR3C2* gene, such as promoter, intronic, 5′ untranslated and/or 3′ untranslated regions.[41-43,45] Mutations in other genes and non-genetic factors are also possible explanations for these cases.[38-43,46,47] Mutations have been detected in all functional domains of MR[1,24] and they are all heterozygous.[36-49] The renal PHA phenotype is due to either haploinsufficiency (loss of one of the two functional alleles) of MR[36,42,44,46,47] or negative dominant effect of the mutated MR on the activity of the wild-type MR.[42] No homozygous MR mutations have ever been detected in humans,[1,9,24] suggesting that the complete absence of both MR alleles, and therefore of MR function, is incompatible with survival in humans.[9,24] Only one case of a compound heterozygote with MR mutations has been reported. When functional studies of these mutations were done, the protein from the one MR gene was degraded, whereas the protein from the other displayed partial activity.[57] On the the other hand, heterozygous for the mineralocorticoid receptor (MR+/-) knockout mice grow and breed normally, whereas only homozygous (MR-/-) knockout mice develop a renal PHA phenotype and die around the tenth day of life from severe dehydration caused by excessive renal sodium and water loss.[58] Nevertheless, these mice can be saved by subcutaneous salt injections until they grow older than a critical age, when they reach a body mass of 8.5 g.[59] MR knockdown rats also develop a renal PHA phenotype.[60] Furthermore, renal PHA is characterized by a highly variable penetrance and expressivity,[34-37,41,42,44-47] this being evident because there are cases of healthy unaffected heterozygotes with normal plasma concentrations of renin and aldosterone,[41,42,44] cases with elevated plasma concentrations of renin and aldosterone, but without electrolyte disturbances or symptoms[34,35,37,40,44,45,47] and patients with renal salt wasting and clinical manifestations.

MR functions as a ligand-induced homodimer and belongs to the nuclear receptor superfamily along with other steroid hormone receptors (glucocorticoid, progesterone, estrogen and androgen receptors) and receptors for thyroid hormone, vitamin D and retinoic acid.[61] Loss-of-function mutations of MR do not permit proper binding and function of aldosterone in the distal renal tubule and therefore sodium reabsorption through ENaC is impaired. Thus, potassium and hydrogen excretion and water reabsorption are also impaired.[9,10,12,28] These changes lead to increased sodium and decreased potassium excretion in urine, hyponatremia, hyperkalemia, metabolic acidosis and volume depletion.[9,10,31] The renin-angiotensin-aldosterone system is also activated on account of the reduction of intravascular volume, resulting in elevated renin plasma levels and elevated aldosterone plasma and urine levels. Aldosterone levels are also increased due to hyperkalemia.[9,12,24,26] Especially in the neonatal period, when renal tubules are immature[1,9,26] and neonates consume human breast milk, which has a low sodium content, the activation of the rennin-angiotensin-aldosterone system needs to be maximal.[9,26] Increased aldosterone plasma concentrations are not sufficient to restore the balance of electrolytes and volume during the neonatal and early infant period, leading to the necessity for salt supplementation and occasionally potassium binding resins.[9] Renal PHA is clinically silent and without any obvious phenotypic consequences in patients on high-salt diet.[9,10] Possible explanations for the improvement of the phenotype after the first years of life are the maturation of the renal tubule,[9,23,24,26] the substitution of the impaired distal sodium reabsorption by sodium reabsorption in proximal parts of the renal tubule,[23,24] the postnatal increase in expression of ROMK,[13,62] the chronic activation of the rennin-angiotensin-aldosterone system, which persists in several adults with renal PHA[9,24,36] and/or the increased salt intake.[9,10,24,26]

SYSTEMIC PHA (AUTOSOMAL RECESSIVE PHA)

Systemic PHA is characterized by systemic resist-

ance to aldosterone that affects numerous organs, such as the kidneys, colon, sweat glands, salivary glands and respiratory tract,[1,9,12,22-26,29] leading to salt loss from the kidneys, colon, sweat and salivary glands.[1,9,23,24,26,29,31] Symptoms begin during the neonatal period[1,9,29,26] and prolonged hospitalizations are necessary.[63] In additon to the other manifestations of PHA, patients sustain recurrent lower respiratory tract infections[63-69] and frequent episodes with symptoms from the lungs, such as cough, wheezing, chest congestion and tachypnea,[67-70] within weeks or months after birth[66-70] and until 5-6 years of age.[63,65,67,69] Sputum cultures from several patients have grown *Staphylococcus aureus, Streptococcus pneumoniae, Pseudomonas aeruginosa, Haemophilus influenzae, Klebsiella pneumoniae, Moraxella catarrhalis* and other bacteria,[66,68] whereas in other cases, sputum cultures have grown only normal flora.[67] However, no patient with systemic PHA has been reported to suffer from neonatal respiratory distress syndrome,[23,26,30,31] with the exception of two newborns.[71,72] Nevertheless, one was a premature (born at 31 weeks of gestation),[72] and it is well known that premature neonates are more susceptible to neonatal respiratory distress syndrome, particularly those born before 34 weeks of gestation,[73] and the other was also a premature, but born at 36 weeks of gestation, and he responded to treatment with fludrocortisone and not to salt supplementation, which is incompatible with PHA diagnosis.[71] Additionally, cases of cholelithiasis,[74,75] skin rashes,[76,77] irritated nasal filtrum area due to chronic discharge of clear liquid from the nose,[67] salt loss from the Meibomian glands of the eye lids[78] and polyhydramnios have been reported.[79-83] The increased sodium levels in sweat and saliva, due to the salt wasting from sweat and the salivary glands, and the absence of nasal and rectal transepithelial potential differences can be useful for the diagnosis of systemic PHA.[1,23,26,31,67,69,84,85] Treatment consists of life-long extensive salt supplementation (20-50 mEq/kg/day of sodium[1]) combined with potassium-binding resins in order to maintain electrolyte and water homeostasis.[1,9-12,22-26,29] Presentation and course of disease are much more severe than those of renal PHA.[1,9,10,12,23-26,28,29] This is a life-long disease[1,10,23,25,26,29] with a generally poor prognosis,[9,24] although there is some variability.[1,23,29] For example, patients with homozygous null mutations often have a very poor prognosis[29] and patients with missense mutations have a milder phenotype.[63,68] There is no remission over time,[1,10,23,24,26] manifestations persist in adults[1,10,23-26,28,29] and patients suffer from recurrent life-threatening episodes of salt loss and hyperkalemia.[9,10,22-26,29,30] Nevertheless, a case of systemic PHA with significant clinical remission and cessation of daily salt supplementation at the age of 11 years has been reported.[86] Additionally, four cases of systemic PHA with clinical improvement after the age of 6-10 years have been reported, but without cessation of treatment. One of them had a constantly mild clinical course.[87]

The underlying cause of systemic PHA is loss-of-function mutations in any gene of the three subunits [alpha (α), beta (β) or gamma (γ)] of ENaC.[63,67-70,88-92] These genes are *SCNN1A* (chromosome 12p13.31), *SCNN1B* (chromosome 16p12.1) and *SCNN1G* (chromosome 16p12.1) for α, β and γ subunits, respectively.[93,94] The mutations are inherited in the autosomal recessive pattern,[63,67-70,88-92] although a sporadic case of a compound heterozygote has also been reported,[70] and include nonsense, missense, frameshift (insertion or deletion) and splice site mutations in the three genes.[63,67-70,88-92] These result in abnormally structured subunits of ENaC with impaired function.[63,67,68,70,88-92] A case of systemic PHA due to a large homozygous deletion in the promoter region of *SCNN1B*, leading to reduced expression of β subunit, has also been reported.[69] Mutations have been detected in the genes of all subunits, but most of them have been found in the gene of the α subunit.[63,67-70,88-92] Patients are either homozygotes[63,67-69,88-92] or compound heterozygotes,[63,67,68,70] but all have both alleles mutated.[63,67-70,88-92] All heterozygote carriers have one mutant and one wild-type allele and they are free of any clinical or laboratory findings.[9,23,92] Nevertheless, increased sodium and chloride concentrations have been found in sweat of heterozygote carriers.[92] Detected mutations are located primarily in the exons of these genes.[63,67-70,88-92] However, there are some cases of systemic PHA in which no mutations in ENaC genes have been detected, probably due to mutations in promoters or introns of ENaC genes or in other genes.[67,88,91] Finally, it has been suggested that some sporadic cases of either renal or systemic form of PHA, in which polymorphisms in the genes of MR and ENaC have been found, could be attributed to the combination of polymorphisms that influence

negatively salt conservation. These polymorphisms alone do not presumably have such a major effect on aldosterone action or sodium handling as to cause clinical or laboratory manifestations of PHA, but when they coexist, their effects aggregate creating a phenotype of PHA.[95,96]

ENaC belongs to the DEG (degenerin)/ENaC gene superfamily[97,98] and is not voltage-gated.[22] It is expressed at the apical membrane of epithelial cells in many tissues (Figure 1), such as in the aldosterone-sensitive distal nephron, distal colon, sweat and salivary glands and the glucocorticoid-sensitive respiratory tract.[1,22,26,27,30,31,98] It is a hetero-multimeric protein composed of three partly homologous subunits, α, β and γ,[99,100] sharing approximately 35% sequence identity.[94] The exact subunit stoichiometry of ENaC remains controversial. Different possible subunit combinations have been proposed: tetrameric (2α:1β:1γ),[101-104] octameric[105] or nonameric (3α:3β:3γ) complex[105-108] or complex with a relative, limiting subunit stoichiometry of 1α:1β:1γ.[109] The three subunits are arranged in a triple-barrel-state,[110] forming together the channel pore,[111,112] on the surface of the cell membrane.[1,22-24,26,27,30] Each subunit consists of two transmembrane regions, a large extracellular loop corresponding to approximately 70% of the protein mass and short intracellular amino- and carboxyl-terminus (with proline-rich regions in the carboxyl-terminus).[1,22,26,98,113] There are two cysteine-rich domains (CRB1 and CRB2), which are involved in the channel trafficking to the cell membrane, and several glycosylation sites in the extracellular loop.[23,98] Lysine residues in the amino-terminal region of the three subunits can be ubiquitinated and are key elements determining the half-life of the channel.[113-117] Moreover, the proline-rich motif (PPPXY) in the intracellular carboxyl-terminus participates in protein-protein interactions and the regulation of the number of ENaCs at the cell membrane.[1,23,98] Missense or deletion mutations within this motif of β or γ subunit have been detected in patients with Liddle's syndrome, which is characterized by hypertension, hypokalemia, metabolic alkalosis and low renin and aldosterone plasma levels.[10,12,23,26,27,29-31,98,118] Moreover, α subunit is required for β and γ subunits to be brought to the cell membrane and β and γ subunits are required for

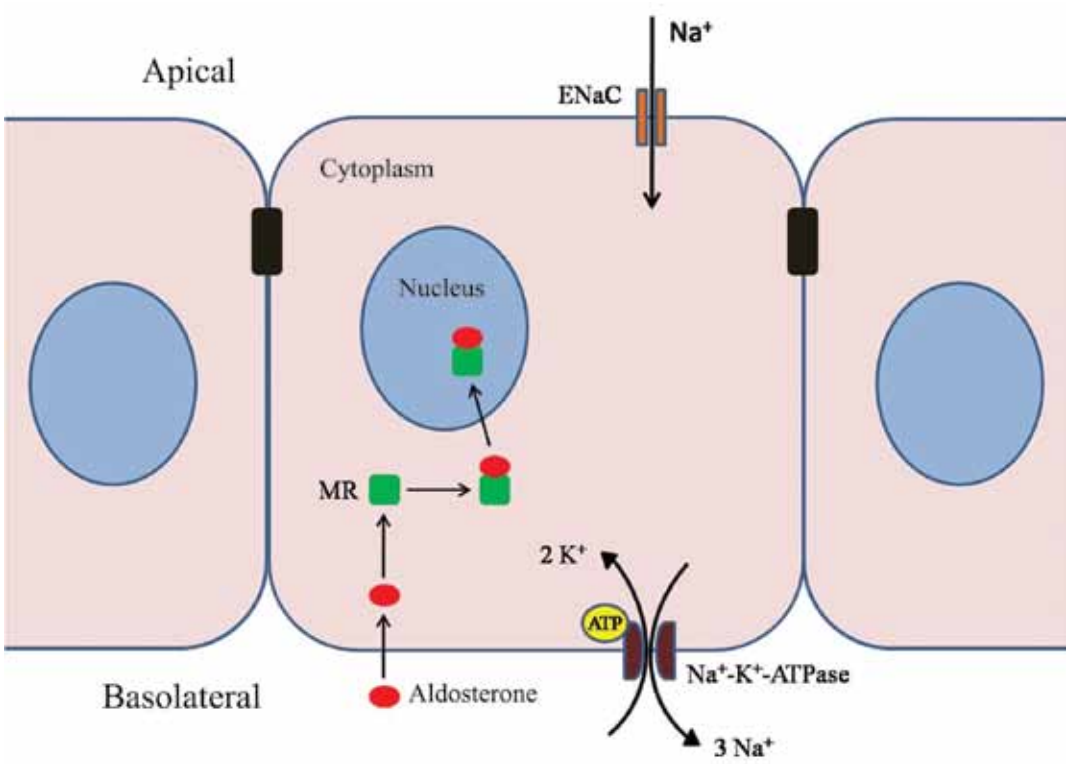

Figure 1. Schematic representation of the sodium reabsorption system in epithelial cells. (MR: mineralocorticoid receptor, ENaC: epithelial sodium channel, Na^+: sodium, K^+: potassium, ATP: adenosine triphosphate).

α subunit to be brought to the cell membrane.[22] The amino-terminal domain of α subunit controls the gating of ENaC.[119-121] ENaC needs all of the three subunits to be fully functional[22,26,27,30,31] and also for its efficient synthesis, assembly and trafficking.[22,27,30] Any disruption of these processes can lead to systemic PHA.[22,26,30] Additionally, a delta (δ)[122] and an epsilon (ε) subunit[123] have been identified. They are partly homologous with α, β and γ subunits and they can replace α subunit, forming ENaCs along with β and γ subunits. Nevertheless, their exact role has not as yet been elucidated.[122-125]

The loss of ENaC function results in impairment of sodium reabsorption in the distal nephron.[9,10,12,29] Thus, potassium and hydrogen excretion and water reabsorption are also impaired. These changes lead to increased sodium and decreased potassium excretion in urine, hyponatremia, hyperkalemia, metabolic acidosis and volume depletion.[9,10,29] The renin-angiotensin-aldosterone system is also activated due to the reduction of intravascular volume, resulting in elevated renin plasma levels and elevated aldosterone plasma and urine levels. Aldosterone levels are also increased due to hyperkalemia.[9,12,22,26,29] Additionally, ENaC participates in fluid reabsorption in glucocorticoid-responsive epithelia, such as at the air-cell interface of proximal and distal airways.[1,10,22,26,27,30,31,98] Thus, the lungs are cleared of fluid at birth and are able to keep dry thereafter.[126] The reduced sodium-dependent liquid absorption from the surface of the respiratory tract in cases of non-functional ENaC results in increased volume of airway surface liquid, more than twice the normal (and as a consequence in increased rate of mucociliary clearance).[67,127] This causes narrowing of airways lumen and/or dilution of stabilizing surface-active materials in small airways, predisposing to respiratory infections and other manifestations from the lungs.[67] An interesting finding is that gain-of-function mutations in ENaC have been observed in patients with a phenotype resembling cystic fibrosis,[128] which is characterized by decreased volume of airway surface liquid and decreased mucociliary clearance.[128-130] Furthermore, the loss of ENaC function also causes salt wasting in sweat, saliva and stool, where sodium and chloride levels are also elevated,[1,9,23,26,31] because patients with systemic PHA present salt loss from all aldosterone-dependent epithelial cells.[63]

The role of the expression of the ENaC subunits has been examined in heterologous expression systems, such as *Xenopus laevis* oocytes, cell lines and mice. The maximal inward current that is produced when different combinations of ENaC subunits are present at the cell surface has been tested in *Xenopus laevis* oocytes. The results have been compared with those of the wild-type ENaC, in which α, β and γ subunits are coexpressed [α subunits only ➔ 1%, α and β subunits only or α and γ subunits only ➔ 10-20%, β and γ subunits only ➔ 2% (only after a long incubation for 6-7 days), β or γ subunits alone ➔ no significant inward current detected].[22,99,131] Moreover, some ENaC mutations found in patients with systemic PHA have been tested in *Xenopus laevis* ocytes[90,119,131,132] and Chinese hamster ovary cells.[133] The activity of ENaCs containing the mutated subunits is significantly reduced.[90,119,131-133] Furthermore, knockout mice for the α subunit (α(-/-)) present reduced mobility, poor appetite and persistent chest wall retraction. They appear to be cyanotic shortly before death, which occurs within 40 hours after birth. Lungs appear to be mottled and full of fluid in autopsy and with higher values of the whole lung wet/dry ratio in comparison with α(+/+) and α(+/-) mice. This happens because ENaC is inactive, as can be concluded from the elimination of amiloride-sensitive potential difference of tracheal cysts, and thus the fluid from the distal airways of the lungs cannot be absorbed, resulting in death due to lung edema. It is therefore evident that α subunit is necessary for fluid absorption in the lungs at birth in mice and β and γ subunits cannot substitute for its role sufficiently.[134] The α(-/-) mice can escape death from lung edema if a transgenic expression of the α subunit is applied by using a cytomegalovirus promoter, thus achieving partial restoration of ENaC activity. These transgenic α(-/-) mice present urinary salt loss and metabolic acidosis within 5-9 days after birth due to the reduced activity of ENaC in kidneys. They also present growth retardation and their mortality is 50%, dying of disturbances in electrolyte homeostasis. Additionally, amiloride-sensitive currents in fetal tracheas and adult airway epithelia of these transgenic α(-/-) mice are slightly, but not significantly, decreased in comparison with those of wild-type mice.[135] Moreover, mice which are heterozygote carriers of mutations in α subunit present

a moderate decrease of extracellular volume, blood pressure, potassium excretion and sodium conservation when salt intake is very low.[134] In contrast, patients with systemic PHA and mutations of α subunit do not suffer from lung edema after birth.[9,10,22,23,26,27,30,31] This indicates that the residual activity of channels consists of β or γ subunits only or alternatively of β and γ subunits, and a truncated α subunit is sufficient to clear the lungs of fluid at birth and prevent the appearance of lung edema afterwards.[26,30,31] Similar findings apply for patients with mutations in β and γ subunits.[9,10,22,23,26,31] Furthermore, knockout mice for β subunit [β(-/-)] or γ subunit [γ(-/-)] die within 48 or 36 hours after birth, respectively, probably of hyperkalemia. They present a renal phenotype with growth retardation, increased sodium and decreased potassium in urine, hyponatremia, hyperkalemia, metabolic acidosis and elevated aldosterone levels, without any major pulmonary manifestations, except for a small but significant increase in lung wet/dry ratio.[136,137] Thus, in humans and mice, a low ENaC activity is enough to clear lungs of fluid after birth and to establish a normal air-liquid interface, but it is not enough to prevent salt loss from the kidneys and keep electrolyte and water homeostasis.[22,26,31] Finally, another interesting finding is that transgenic mice overexpressing wild-type β subunit develop a cystic fibrosis-like lung disease because airway surface liquid volume is depleted and the mucus clearance is insufficient due to the increased sodium reabsorption through ENaC, inducing airway obstruction from mucus.[138-140]

SECONDARY PHA

Secondary PHA is a form of renal resistance to aldosterone. The underlying cause can be either renal diseases or medications. The clinical and laboratory findings resemble those of a transient PHA.[1,9,11,26,141] Secondary PHA may occur mainly in neonates and young infants with urinary tract infections, such as pyelonephritis, and/or malformations of urinary system causing obstructive uropathy, such as ureteropelvic junction obstruction, ureterovesical junction obstruction, ureterocele and posterior urethral valves.[1,9,23,24,26,141] There is a preponderance of male infants due to the higher incidence of urinary tract infections and obstructive uropathy in them rather than in female infants.[141] Patients present hyponatremia, hyperkalemia, metabolic acidosis, increased sodium and decreased potassium excretion in urine, and elevated levels of renin in plasma and aldosterone in plasma and urine, reflecting renal resistance to aldosterone, along with vomiting, diarrhea, polyuria, dehydration, insufficient weight gain and failure to thrive.[9,26,141] Acute deterioration of patients' condition with severe weight loss, peripheral circulatory failure, reduction of GFR (in contrast to PHA and FHH), demonstrated by increased urea and creatinine serum levels and decreased creatinine clearance, and/or life-threatening hyperkalemia may also occur.[23,26,141] Therapy involves medical or surgical treatment of the underlying cause and volume resuscitation. In severe cases, supplemental sodium (3-20 mEq/kg/day, given as sodium chloride or sodium bicarbonate) is also administered.[1,141] The clinical and laboratory findings improve within one or two days and disappear after the completion of medical treatment of urinary tract infection and/or surgical correction of obstructive uropathy, usually within a few days to one week after the beginning of treatment.[9,24,141] Nevertheless, patients with permanent renal damage need prolonged sodium bicarbonate administration.[141] Generally, severe manifestations occur in patients up to three months old, whereas the risk of their appearance is greatly decreased after this age and disappears after the age of seven months.[1,141] Thus, only normokalemic secondary PHA has been reported in older children with pyelonephritis, presented during the first few days, with normalization of findings within three weeks' time.[141]

Concerning the underlying pathophysiology, it has been found that the intrarenal expression of several cytokines, such as tumor necrosis factor alpha (TNF-α), interleukin (IL)-1, IL-6, transforming growth factor beta-1 (TGF-β1), angiotensin II, endothelin, thromboxane A2 and prostaglandins, is increased in cases of obstructive uropathy. Furthermore, it has been found that the intrarenal synthesis of prostaglandins, thromboxane, leukotriens, endothelin and IL-1 is increased under the influence of bacterial endotoxin in cases of urinary tract infections. These changes result in inhibition of aldosterone action through reduction of its expression and/or impairment of its receptor, vasoconstriction and reduction of GFR, increased

natriuresis and/or decreased Na^+-K^+-ATPase activity.[26,141] Finally, some patients with secondary PHA phenotype may also develop salt wasting from the intestinal tract (for example, after major small bowel resection) or sweat glands.[1,9,23,24,26]

In addition, there are a number of drugs which may cause resistance to aldosterone, and therefore secondary PHA.[1,9,11,141] Indeed, there are medications that block ENaC, such as amiloride, triamterane, trimethoprim and pentamidine, or MR, such as spironolactone. Furthermore, there are medications that interfere with the renin-angiotensin-aldosterone system, like the angiotensin converting enzyme (ACE) inhibitors and the angiotensin II receptor antagonists (especially in cases of coexisting renal insufficiency or diabetic nephropathy), the non-steroidal anti-inflammatory drugs (NSAIDs) (cycloxygenase inhibitors) (inhibition of renin release), heparin (inhibition of aldosterone synthase - 18-hydroxylase) which leads to impairment of aldosterone synthesis, and lastly the beta-adrenergic antagonists which decrease Na^+-K^+-ATPase activity and inhibit renin release.[11,141] Additionally, the calcineurin inhibitors, such as cyclosporine A and tacrolimus, may also lead to resistance to aldostrone.[1,9,11,141] Possible mechanisms that have been proposed include: 1. inhibition of Na^+-K^+-ATPase activity, 2. vasoconstriction, which decreases GFR and the filtered potassium load,[11] 3. inhibition of renin[142] and aldosterone secretion,[142,143] 4. reduction of MR expression,[144,145] 5. impairment of MR transcriptional activity.[146]

FAMILIAL HYPERKALEMIC HYPERTENSION (FHH)

FHH is a rare heterogeneous syndrome inherited in the autosomal dominant pattern.[147-158] It is characterized by hypertension, hyperkalemia, hyperchloremia, metabolic acidosis, low potassium renal clearance, hypercalciuria, low plasma renin levels and highly variable plasma and urine aldosterone levels, but usually almost normal, however lower than would be expected considering the hyperkalemia. Adrenal function and GFR are normal.[2-4,9-21,23,26,29,32,118,159] Low bone mineral density (BMD) is also found in patients with hypercalciuria due to urinary calcium loss.[149,158] Symptoms and laboratory findings begin in childhood,[159] but hypertension may manifest later in life, usually ten to twenty years later than the onset of the other manifestations and between the second and the fourth decade of life.[149,153,155,158,160] It should be noted here that only half of the patients with FHH are hypertensive at the time of diagnosis.[15] However, all patients eventually develop hypertension during their adult life if they are left untreated.[159] A possible explanation for the variability in the onset of hypertension in patients with FHH is the variability in dietary salt intake among them.[15,18] Moreover, there are large variations with regard to age of discovery and the severity of manifestations among the patients and the families with FHH.[153] Hyperkalemia and low potassium renal clearance remain when sodium chloride is infused intravenously, but intravenous infusion of sodium sulfate (a non-chloride sodium salt) increases renal clearance of potassium and lowers potassium levels in serum.[15,19,21,159] Interestingly, most patients in whom endogenous or exogenous mineralocorticoids have been administered exhibited no resistance to them.[147] Chronic dietary salt intake restriction and per os administration of low doses of thiazide diuretics, which inhibit NCC activity, are effectively used as treatment of FHH and correct the clinical and laboratory manifestations of this syndrome.[2,4,9,11,12,14-21,23,26,32,118,159,161]

FHH is heterogeneous and 4 loci have been associated with the trait: a) 1q31-42, b) 17p11-q21, c) 12p13.3 and d) an as yet unidentified locus. FHH mutations in these loci are inherited in the autosomal dominant pattern.[150-152,155] The second and the third locus include the WNK4 (17q21.31) and the WNK1 (12p13.33) gene, respectively.[21,154,162,163] WNK1 and WNK4 are members of the WNK [with-no-lysine (K) kinase] family, which consists of four serine-threonine protein kinases: WNK1, WNK2, WNK3, WNK4,[3,4,14,15,17,20,21,159,161,164] whose genes *PRKWNK1, PRKWNK2, PRKWNK3, PRKWNK4* are located on chromosomes 12p13.33, 9q22.31, Xp11.22, 17q21.31, respectively.[21,162,163] Their name is due to the atypical placement of their catalytic lysine in the kinase domain since all the other protein kinases have a conserved catalytic lysine in sub-domain 2 of the kinase domain, which is critical for ATP binding in the catalytic site, whereas the catalytic lysine of WNKs is located in sub-domain 1 and is substituted

by cysteine in sub-domain 2.[3,4,14-18,20,21,159,161,164] WNKs share a 40% sequence identity (85-90% in the kinase domain and <20% in the other domains).[4,14,15,20,21,159] Apart from their kinase domain, which is located in the amino-terminal part, they also have some other highly conserved domains in their carboxyl-terminal part which are important for protein-protein interactions: a) an autoinhibitory domain, which is located between the kinase domain and the first coiled-coil domain and binds to the kinase domain of the same or other WNK, inhibiting its activity, b) two coiled-coil domains and c) multiple PXXP proline-rich motifs which interact with SH3 domains of other proteins (Figure 2).[2-4,14-18,20,21,26,159,161,164] Moreover, WNKs have a very short and highly conserved segment of ten amino-acid residues that is negatively charged and is located just distal to the first coiled-coil domain.[4,20] WNKs have little sequence identity in the other domains.[14,15,17,20] WNKs can interact with each other by either phosphorylation or protein-protein interactions.[2,4,20,21] WNKs are expressed in many tissues, they have various splice forms among different tissues and they are involved in ion balance, proliferation, survival, cell signaling and organ development.[2-4,14-17,20,21,29,159] The expression of WNK1 is widespread. It has been detected in tissues, such as kidneys, testes, epididymides, heart, skeletal muscles, lungs, placenta, brain, colon, small intestine, esophagus, pancreas, liver, gallbladder, spleen, adrenals, skin, sweat ducts and macrophages.[162,165-168] The expression of WNK4 has been detected in kidneys, testes, epididymides, heart, lungs, brain, liver, pancreas, colon, prostate, skin and sweat ducts, presenting a more limited expression pattern.[162,169] Among the tissues where WNK1 and WNK4 are expressed, there are many polarized epithelia involved in chloride ion transport.[165,169] In kidneys, WNK1 and WNK4 are found primarily in the distal nephron.[154,165,169,170] WNK1 is localized in the cytoplasm of epithelial cells of DCT and the collecting duct (CD), whereas WNK4 is localized in the tight junctions of DCT and in the cytoplasm of epithelial cells and the tight junctions of CCD.[154,165,169] WNK4 is also expressed in TAL and expression of WNK1 is detected in the entire nephron.[170] WNK1 has several isoforms due to alternative slicing variants.[167,168,171] The longest isoform of WNK1, which is expressed in the entire nephron and in all the other tested tissues, is derived from two transcripts, which have a different

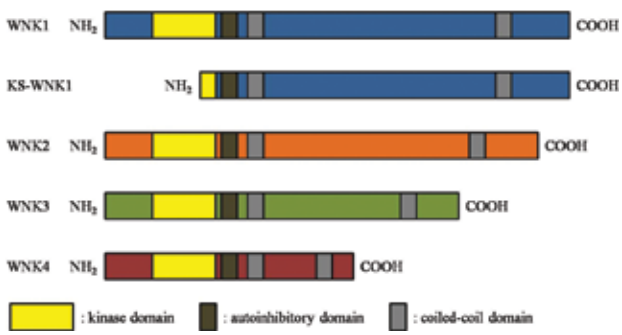

Figure 2. Schematic representation of the structure of WNK kinases. [WNK: with-no-lysine (K) kinase; KS: kidney-specific; NH_2: amine group; COOH: carboxylic acid group].

length of the 3´ untranslated region due to different polyadenylation sites.[167,168] A shorter kidney-specific isoform of WNK1 (KS-WNK1) is derived from alternative splicing of exons 1-4 and is the most prominent isoform of WNK1 in kidneys. Alternative splicing of exons 9, 11 and 12, separately or combined, produces five more different transcripts, but the functions of the corresponding isoforms are unknown.[167,168,171] A large number of studies on WNK4 and WNK1 have been conducted in heterologous expression systems, such as *Xenopus laevis* oocytes, cell lines, mice and rats.

WNK4 is involved in several sites of ion transport in the distal nephron. The pathophysiological mechanisms are as follows: Firstly, it decreases NCC activity by reducing the amount of NCCs at the cell membrane[156,157,172-184] through their increased degradation in lysosomes,[177,178,181,182] probably after phosphorylation of NCC by WNK4.[177,182] Two members of the Ste20p family, SPAK (Ste20-related proline and alanine-rich kinase) and OSR1 (oxidative stress responsive kinase-1), which phosphorylate NCC, may be involved in the regulation of NCC by WNK4.[185,186] It has been found that in transgenic mice with WNK4 mutations, mutated WNK4 increases the cell surface expression of NCC via the increased phosphorylation of SPAK and OSR1.[185] On the other hand, the phosphorylation of OSR1/SPAK and NCC is decreased in WNK4 hypomorphic mice.[186] Moreover, WNK4 phosphorylates claudins 1-4 and 7 in tight junctions, increasing paracellular chloride reabsorption.[187-189] Furthermore, WNK4 reduces ROMK activity[156,174,184,190-194] by interacting with intersectin. This leads to stimulation of ROMK endocytosis via clathrin-coated vesicles, which

results in a decreased amount of ROMKs at the cell membrane.[174,191] After the kinase domain and exactly before the acidic region where several FHH mutations of WNK4 occur, there is a cluster of PXXP motifs which are important for binding of WNK4 to SH3 domains of intersectin. It has also been suggested that mutations of WNK4 cause changes of PXXP motifs conformation, enhancing the interaction between WNK4 and intersectin, which results in increased ROMKs endocytosis via clathrin-coated vesicles.[191] Additionally, WNK4 inhibits ENaC activity,[190,195] presumably by increasing its ubiquitination and endocytosis through the E3 ubiquitin ligase Nedd4-2 (Neural precursor cell Expressed, Developmentally Down-regulated 4-2), which acts via a clathrin-dependent mechanism. This results in a decreased amount of ENaCs at the cell membrane.[27,195] On the other hand, it has also been reported that WNK4 increases ENaC expression on the cell surface, and therefore its activity, by activating SGK-1 (serum- and glucocorticoid-induced protein kinase 1) via indirect induction of its phosphorylation. SGK-1, in turn, phosphorylates and deactivates Nedd4-2, preventing endocytosis of ENaC.[196] WNK4 also increases Na^+–K^+–$2Cl^-$ cotransporter (NKCC) 1 and 2 activity (reabsorption of sodium, potassium and chloride). This action is mediated by SPAK and OSR1, which bind to NKCC and poshporylate it.[197-202] (NKCC2 is located at the apical membrane of epithelial cells of TAL and macula densa.[203] NKCC1 is ubiquitously expressed[3,21,164] and is located at the basolateral membrane of the outer medullary collecting duct in kidneys.[204,205] NKCC1 is also important for contraction of vascular smooth muscle cells.[14] Nevertheless, there is data suggesting that WNK4 decreases the cell surface expression of NKCC1 and therefore its activity.[169] Transgenic mice with FHH-WNK4 mutations have reduced expression and activity of NKCC2.[183] WNK4 also reduces the activity of K^+-Cl^- cotransporter (KCC) 1-4[197,206] (excretion of potassium and chloride), which are located in the nephron (proximal tubule (PT), TAL, DCT, CD) and other tissues,[3,4,14,206] and inhibits the activity of Cl^-/base exchanger CFEX, which is located at the apical membrane of epithelial cells of PT and other tissues.[169] Apart from the above, WNK4 enhances calcium uptake through the TRPV5 (transient receptor potential, vanilloid subfamily, member 5) channel,[207-209] which is located at the apical membrane of DCT-2 and CNT,[7,210] by increasing its cell surface expression, though it does not affect significantly calcium uptake through the TRPV6,[207-209] which is located at the apical membrane of DCT-2, CNT and CD.[210] Nevertheless, there is evidence that WNK4 interacts with intersectin, leading to stimulation of caveola-mediated endocytosis of TRPV5 and thus decreasing its cell surface expression and therefore its activity.[211] WNK4 also inhibits calcium entry through the TRPV4 channel,[212] which is located at the basolateral membrane of the nephron distally to the junction of the descending and the ascending limb of Henle's loop (apart from the macula densa),[213] by decreasing its cell surface expression, although its total expression in cells is unaffected.[212] Coexpression of NCC with TRPV5 in *Xenopus laevis* oocytes reduces the effect of WNK4 on TRPV5 in a dose-dependent manner.[207] Finally, it has recently been reported that WNK4 inhibits Maxi-K potassium channel activity by reducing its presence at the cell membrane through enhanced lysosomal degradation.[214] WNK4 regulates NCC,[172,177,178] chloride flux in tight junctions,[187-189] NKCC,[197,199,201] KCC,[197,206] TRPV4,[212] TRPV5[207] and the Maxi-K potassium channel[214] via catalytic mechanisms (depending on its kinase activity) and ROMK[174,191,192] and ENaC[195,196] via non-catalytic mechanisms (protein-protein interactions), though there is evidence that non-catalytic mechanisms may also be involved in regulation of NCC[176,186] and TRPV5.[211]

WNK1 is involved in many sites of ion transport in the distal nephron. Firstly, it increases the activity of NCC by inhibiting the inhibitory effect of WNK4 and therefore increasing NCC expression at the cell membrane, thus having an indirect effect on NCC.[173,176,178,215] WNK1 inhibits WNK4 by phosphorylating it,[176,180,216] although there are data suggesting that the regulation of WNK4 is not dependent on the catalytic activity of WNK1.[178] WNK1 has no effect on NCC activity when WNK4 is absent.[173] Nevertheless, there is evidence that WNK1 activates SPAK and OSR1, which phosphorylate NCC and increase its activity.[217,218] Moreover, there are indications that WNK1 phosphorylates claudin 4, a protein of tight junctions, and increases paracellular chloride permeability.[219] Furthermore, WNK1 reduces the activity of ROMK[191,220-226] by interacting with intersectin. This leads to stimulation of ROMK endocytosis through clathrin-coated

vesicles,[191,220,223,224,226] resulting in a decreased amount of ROMKs at the cell membrane.[191,220-226] There are PXXP motifs in the amino-terminal part of WNK1, before the kinase domain, which are important for binding of WNK1 to SH3 domains of intersectin. It has also been found that the conformation of WNK1 kinase domain, but not its kinase activity, affects the interaction of these PXXP motifs with the SH3 domains of intersectin.[191,224] Additionally, WNK1 enhances ENaC activity by activating SGK-1 via indirect induction of its phosphorylation. SGK-1, in turn, phosphorylates and inhibits Nedd4-2. This inhibits ubiquitination and endocytosis of ENaC via a clathrin-depended mechanism and therefore results in an increased amount of ENaCs at the cell membrane.[27,196,227,228] Apart from the above, WNK1 increases NKCC1 and NKCC2 activity through SPAK and OSR1[201,217,229,230] and reduces KCC3 activity.[231] It also inhibits calcium entry through the TRPV4 channel by decreasing its expression on the cell surface via a mechanism moderately dependent on kinase activity of WNK1, while its total expression in cells is unaffected.[212] On the other hand, WNK1 does not increase calcium entry through TRPV5 or TRPV6 significantly.[207] WNK1 regulates NCC,[176,180,216,217] chloride flux in tight junctions,[219] NKCC[201,217,229,230] and probably KCC[231] via catalytic mechanisms (depending on its kinase activity) and ROMK[191,223,224] and ENaC[196,228] via non-catalytic mechanisms (protein-protein interactions), even though there is evidence that non-catalytic mechanisms may also be involved in NCC regulation[178] and catalytic mechanisms in ROMK regulation.[220,221]

There is a shorter kidney-specific isoform of WNK1 (KS-WNK1), as has been mentioned previously, which is the most prominent isoform of WNK1 in the kidneys. It is expressed only in the distal nephron, mainly in DCT and CNT and with somewhat reduced expression in CCD, and in no other tissues.[167,168,170] It lacks 437 amino acid residues from the amino-terminal edge of WNK1, which include almost the entire kinase domain, due to alternative splicing of exons 1-4, resulting in an isoform without protein kinase activity[167,168,171] that antagonizes the effects of WNK1 on NCC and ROMK, acting as a dominant negative regulator of it.[215,220-222] KS-WNK1 also stimulates ENaC activity.[242] This isoform has an alternative promoter located in intron 4 of *PRKWNK1* and contains sequences from an extra exon, termed 4a, which replaces exons 1-4, resulting in replacement of the 437 amino acid residues of the amino-terminal edge with a cystein-rich region of 30 amino acid residues.[167,168,171] The WNK1/KS-WNK1 ratio in the distal nephron is about 15% WNK1 versus 85% KS-WNK1,[167,168,220] making KS-WNK1 more abundant than WNK1.[167,168,170]

In cases of FHH, the mutations in *PRKWNK1* and *PRKWNK4* genes do not affect the protein kinase domain of WNK1 and WNK4, respectively.[154,156,157,232,233] WNK1 mutations are large deletions in the first intron of the gene, which lead to a fivefold enhanced expression of the wild-type protein (as can be concluded by the fivefold increased levels of WNK1 mRNA in leukocytes of patients with WNK1 mutations).[154] This probably increases the WNK1/KS-WNK1 ratio and thus enhances the effects of WNK1.[4,17,21] Nevertheless, it has been found that deletion in the first intron of *PRKWNK1* in a transgenic mouse model results in overexpression of both isoforms (long WNK1 and KS-WNK1) in DCT and ubiquitous ectopic expression of KS-WNK1. The expression of KS-WNK1 is also increased in the leukocytes of these mice, whereas that of long WNK1 is not, leading to the conclusion that the overexpression of WNK1 in leukocytes of patients with FHH may be attributed to the ectopic expression of KS-WNK1 only.[234] WNK4 mutations are missense mutations that take place in the coding sequence of the gene, outside and after the protein kinase domain.[154,156,157,232,233] They have been detected mostly in a highly conserved negatively charged segment (acidic region) of ten amino-acid residues (six out of ten are charged[4]) just distal to the first coiled-coil domain,[154,156,232,233] or less frequently close to the carboxyl terminus, just distal to the second coiled-coil domain[154] or within it.[157] Most of the WNK4 mutations change the charge and all change the type of an amino acid residue,[154,156,157,232,233] altering the tertiary structure and/or the net charge of WNK4. These changes affect its protein kinase activity and its interactions with other proteins.[4,18,159] Mutations in only one of the two alleles of WNK1 or WNK4 are enough to produce FHH phenotype and therefore FHH is inherited in the autosomal dominant pattern.[159]

The pathophysiology of hypertension in FHH includes changes in NCC and ENaC activity and

paracellular chloride permeability. NCC expression on the cell surface and therefore its activity are increased, leading to enhanced reabsorption of sodium and chloride. This happens because the mutated WNK4 fails to inhibit NCC expression on the cell surface,[156,157,172-174,177-180,183-185] whereas the overexpressed WNK1 causes a greater inhibition of WNK4 inhibitory effect on NCC.[173,176,215] The overexpression of WNK1 may also lead to enhanced phosphorylation and activation of SPAK and OSR1, resulting in turn in further phosphorylation and activation of NCC.[217,218] Moreover, paracellular chloride reabsorption through tight junctions is further increased. This happens because the mutations of WNK4 result in a further enhanced phosphorylation of claudins 1-4 and 7,[187-189] whereas the overexpressed WNK1 also induces a further enhanced phosphorylation of claudin 4.[219] Sodium reabsorption is also increased secondarily.[3,14,15,17,21,159,235] Additionally, ENaC expression on the cell surface and therefore its activity are increased via regulation of the SGK-1 and Nedd4-2 pathway,[195,196,227] leading to enhanced reabsorption of the remaining sodium. This happens because the WNK4-induced inhibition of ENaC activity is prevented by WNK4 mutations,[190,195] whereas the overexpression of WNK1 results in a further increase of ENaC activity.[196,227] These changes cause hypertension because the increased salt and water reabsorption causes expansion of intravascular volume.[12,14-18,20,21,159] However, the degree of contribution of each mechanism is not the same between cases of mutated WNK4 and cases of overexpressed WNK1. First of all, hypertension in patients with mutated WNK4 is much more sensitive to treatment with thiazide diuretics than in patients with essential hypertension.[149,156] This data combined with similar data from mice with mutated WNK4[185] leads to the conclusion that the increased NCC activity is the major cause of hypertension in cases of mutations in the WNK4 gene and plays a more important role than the enhanced paracellular chloride reabsorption[14-17] and the increased ENaC activity.[19,161,185] The last may also be the outcome of a compensatory response of the distal renal tubule to the reduced sodium delivery and fluid flow after DCT because, although the expression of ENaC is increased in transgenic mice with FHH-WNK4 mutations, this is not evident after hydrochlorothiazide treatment.[185] By contrast, in cases of WNK1 overexpression, hypertension is not particularly sensitive to treatment with thiazide diuretics, as there are hypertensive patients with WNK1 overexpression under thiazide treatment who have arterial blood pressure higher than 140/90 mmHg.[152] This indicates that perhaps increased NCC activity does not contribute to the mechanism of hypertension in cases of overexpressed WNK1 to the same extent as in cases of mutated WNK4. A possible explanation is that the increased activity of ENaC also contributes to a significant degree to pathogenesis of hypertension in cases of WNK1 overexpression and hypertension in these patients can be attributed to the increased activity of both NCC and ENaC.[3,14,15,17,236]

Concerning hyperkalemia in FHH, it has been found that reduced urinary potassium excretion and hyperkalemia are due to the further increased inhibition of ROMK expression on the cell surface and thus of its activity, through acceleration of ROMK endocytosis via the clathrin-coated pathway,[174,191,220,223,224] by mutated WNK4[156,174,184,190,191] or overexpressed WNK1.[191,220-224] Nevertheless, no difference in the apical localization of ROMK in the distal nephron between transgenic mice with WNK4 mutations and wild-type mice has been found. This leads to the hypothesis that a relative deficiency of ROMK expression exists and ROMK expression is not increased despite hyperkalemia.[179,185,237] Hyperkalemia is also the result of the decreased amount of the available sodium for exchange for potassium in CNT and CCD, due to the increased sodium reabsorption through NCC in DCT, the previous part of the renal tubule, and the consequential less negative potential in CNT and CCD lumen, which drives less potassium[2,3,14,16,18-21,161] and hydrogen in it.[3,20] The less negative luminal potential and the reduced fluid flow in CNT and CCD, due to the increased salt reabsorption through NCC in DCT, explain why hyperkalemia is not corrected despite the compensatory threefold increase of maxi-K potassium channels expression.[185] Another reason for the less negative luminal potential of CNT and CCD, and therefore the less potassium and hydrogen excretion in it, is the increased chloride reabsorption through NCC and the paracellular pathway, which leaves less chloride in the lumen of more distal parts of the renal tubule (chloride shunt hypothesis).[15,21,159] Furthermore, hyperkalemia and decreased urinary hydrogen excretion result in metabolic acidosis.[15,20,159]

Finally, contrary to the differences in response of hypertension to treatment between patients with mutated WNK4 and overexpressed WNK1, hyperkalemia is sensitive to treatment with thiazide diuretics in both types.[149,152,156,185]

Moreover, the increase of blood pressure due to the intravascular volume expansion cause inhibition of renin expression, resulting in low plasma renin levels[11,12,14-17,118,159] in all patients, hypertensive and normotensive.[158,160,238] Plasma and urine aldosterone levels are highly variable, but usually almost normal, however lower than would be expected considering the hyperkalemia,[2,3,9,23,26,159] since, although typically the lower plasma renin levels would lead to lower plasma aldosterone levels,[11,159] hyperkalemia stimulates aldosterone secretion.[3,4,12,16,18,20,118,159] Thus, these two opposite stimuli are balanced, resulting in almost normal plasma and urine aldosterone levels.[159]

Additionally, patients with WNK4 mutations present mild to moderate hypercalciuria,[149,158,238] which is more sensitive to treatment with thiazide diuretics than hypercalciuria of other patients without FHH.[149] A possible mechanism of hypercalciuria is the enhanced NCC activity,[5,15,149,179,183,207,211,239] which leads to increased entry of sodium and chloride in the epithelial cells of DCT. This results in increased intracellular sodium concentration and therefore cell depolarization. The consequent decreased calcium entry into the epithelial cells of DCT through their apical membrane reduces calcium reabsorption via voltage-sensitive calcium channels. Thus, calcium excretion in urine is increased, causing hypercalciuria.[5,239] It has also been found that calcium uptake through the TRPV5 channel is reduced when NCC activity is enhanced, although mutant WNK4 retains its ability to enhance TRPV5 activity to a similar extent with wild-type WNK4[207] and no significant difference in the expression of TRPV5 in the distal nephron is detected between transgenic mice with WNK4 mutations and wild-type mice.[183] Nevertheless, there is also evidence that WNK4 mutations enhance the inhibition of TPRV5 activity through reduction of its cell surface expression via increased caveola-mediated endocytosis. The deceased calcium reabsorption through TRPV5 and the consequent increased urinary calcium loss may also contribute to the development of hypercalciuria in patients with WNK4 mutations.[211] Moreover, WNK4 mutations reduce the inhibitory effect of WNK4 on TRPV4 activity.[212] It can be assumed that the increased calcium entry through TRPV4 at the basolateral membrane may lead to cell depolarization and decreased calcium entry through the apical membrane from the lumen of the distal renal tubule. On the other hand, transgenic mice with WNK4 mutations have increased TRPV6 expression in the apical membrane of the distal nephron, which may be a compensatory response to the reduced calcium reabsorption and hypercalciuria,[183] since WNK4 does not affect significantly calcium uptake through TRPV6.[207-209] Another possible mechanism of hypercalciuria is that the increased distal sodium reabsorption causes decreased proximal reabsorption of sodium, lithium and calcium, resulting in hypercalciuria.[238] In contrast, patients with overexpressed WNK1 have normocalciuria.[160] This finding suggests again that the increase of NCC activity does not contribute to the pathophysiology of their disease to the same extent as in patients with WNK4 mutations and that the increased activity of ENaC plays a major role in cases of WNK1 overexpression.[15]

Some additional interesting findings come from studies in mice. Transgenic mice with WNK4 mutations present hyperplasia of DCT along with increased NCC expression, whereas transgenic mice with wild-type WNK4 present hypoplasia of DCT along with decreased NCC expression. In double-mutant mice (mice with mutated WNK4 and deficiency of NCC), the FHH phenotype and the hyperplasia of DCT do not appear.[179] Thiazide administration corrects FHH phenotype in mice with WNK4 mutations.[179,185] On the other hand, WNK1 is important for embryogenesis, this being evident from the fact that mice homozygous for WNK1 deletion die before the thirteenth day of gestation. However, mice heterozygous for WNK1 deletion express approximately half of the WNK1 mRNA amount in comparison with normal mice and survive to adulthood; their arterial blood pressure is moderately decreased, but without any detectable abnormalities of levels of electrolytes or other biochemical parameters in blood or urine.[240]

Finally, WNKs regulate ion handling in the distal nephron according to blood volume and potassium serum levels. During hyperkalemia, the elevated aldosterone levels lead to stimulation of SGK-1,

which phosphorylates WNK4 and inhibits its action on NCC, ENaC and ROMK, increasing their activity.[190,241] The elevated aldosterone levels also result in increased expression of KS-WNK1,[241-243] whereas the expression of long WNK1 is not affected, leading to increased activity of ENaC and ROMK and eventually decreased activity of NCC.[241,242] Thus, potassium excretion is increased without increased salt retention because salt reabsorption by NCC in DCT is inhibited, leaving enough sodium in CNT and CCD to be exchanged with potassium through the ENaC-mediated sodium reabsorption and the conjugated ROMK-mediated potassium excretion, which are augmented.[190,241,242] During hypovolemia, WNK4 is converted into a FHH-like state, enhancing NCC and ENaC activity and paracellular chloride flux and reducing ROMK activity and therefore leading to increased salt reabsorption without potassium wasting.[190,241] A possible mechanism for this molecular switch could be the increased levels of angiotensin II due to the activation of the renin-angiotensin-aldosterone system in hypovolemia, which lead to increased levels of cytosolic calcium (Ca^{2+}) and its possible enhanced binding to the negatively charged segment of ten residues of WNK4, where many FHH mutations occur.[20,190]

Furthermore, high dietary potassium intake enhances WNK4 expression, whereas low dietary potassium intake does not affect it significantly.[170] Low dietary potassium intake also increases the WNK1/KS-WNK1 ratio, leading to reduction of ROMK activity and enhancement of ENaC and NCC activity, which result in decreased potassium excretion and increased sodium reabsorption. On the other hand, high dietary potassium intake decreases the WNK1/KS-WNK1 ratio, leading to enhancement of ROMK activity and reduction of ENaC and NCC activity, which result in increased potassium excretion and decreased sodium reabsorption. Thus, when dietary potassium intake is low, kidneys conserve potassium and sodium simultaneously and when it is high, they excrete potassium and sodium simultaneously.[170,220,221] Additionally, KS-WNK1 expression presents a marginally significant reduction in low sodium dietary intake in comparison with high sodium dietary intake and is enhanced under the influence of chronically elevated aldosterone levels.[170]

Actions of WNK4 and WNK1 with and without mutations on NCC, paracellular chloride permeability, ROMK and ENaC are compared in Table 1.

PHA and FHH are compared in Table 2.

CONCLUSIONS

PHA and FHH are rare heterogeneous syndromes. Physicians, especially pediatricians, need to consider PHA as a possible underlying cause in neonates or infants with normal GFR and adrenal function who present manifestations of PHA and do not respond to administration of exogenous mineralocorticoids. The distinction between the renal and the systemic form of PHA is based on the presence of renal salt loss alone or combined with salt loss from sweat and salivary glands and colon, respectively. FHH should be suspected in cases of children with normal GFR and adrenal function who present manifestations of FHH and respond to per os administration of low doses of thiazide diuretics. A medical family history of PHA or FHH increases the likelihood of diagnosis. Finally, secondary PHA must be taken into consideration in neonates or infants with urinary tract infections and/or obstructive uropathy combined with reduced GFR and manifestations similar to those of PHA.

The discovery of the underlying pathophysiology in PHA and FHH has revealed several mechanisms involved in the renin-angiotensin-aldosterone system and the regulation of reabsorption and excretion of

Table 1. Actions of WNK4 and WNK1 with and without mutations on NCC, paracellular chloride permeability, ROMK and ENaC. (The number of the arrows does not represent the degree of the action. A double arrow represents augmentation of an action of the FHH WNK in comparison with that of its wild-type form).

	Wild-type WNK4	FHH WNK4	Wild-type WNK1	FHH WNK1
NCC	↓	↑	↑	↑↑
Paracellular chloride permeability	↑	↑↑	↑	↑↑
ROMK	↓	↓↓	↓	↓↓
ENaC	↓	↑	↑	↑↑

WNK: with-no-lysine (K) kinase; FHH: familial hyperkalemic hypertension; NCC: Na^+-Cl^- cotransporter; ROMK: renal outer medullary potassium channel; ENaC: epithelial sodium channel.

Table 2. Comparison between PHA and FHH.

	Renal PHA	Systemic PHA	Secondary PHA	FHH
Type of inheritance	Autosomal dominant	Autosomal recessive	Not inherited	Autosomal dominant
Mutated gene	*NR3C2* (4q31.1)	a) *SCNN1A* (12p13.31), b) *SCNN1B* (16p12.1), c) *SCNN1G* (16p12.1)	-	a) 1q31-42, b) *PRKWNK4* (17q21.31), c) *PRKWNK1* (12p13.33), d) unidentified locus
Mutated protein	MR	ENaC (subunit α, β or γ)	-	a) Unidentified, b) WNK4, c) WNK1, d) unidentified
Age of onset	Neonatal or early infant period	Neonatal period	Neonatal or early infant period	Childhood
Salt loss/retention	Salt loss (kidneys)	Salt loss (kidneys, colon, sweat glands, salivary glands, manifestations from lungs)	Salt loss (kidneys)	Salt retention (kidneys)
Blood pressure	Hypotension	Hypotension	Hypotension	Hypertension
Renin levels	↑	↑	↑	↓
Aldosterone levels	↑	↑	↑	Variable (usually almost normal)
GFR	Normal	Normal	↓	Normal
Duration of manifestations and treatment	Until early childhood	Life-long	Up to a few days to one week after the initiation of treatment	Life-long
Prognosis	Good	Poor	Good	Good

PHA: pseudohypoaldosteronism; FHH: familial hyperkalemic hypertension; MR: mineralocorticoid receptor; ENaC: epithelial sodium channel; WNK: with-no-lysine (K) kinase; GFR: glomerular filtration rate.

ions in the distal nephron. WNK4 and WNK1 kinases in particular are two protein kinases whose role in blood pressure control and renal ion handling has recently been clarified, rendering them potential targets for future antihypertensive drugs. The elucidation of the underlying mechanisms of PHA and FHH is thus capable of contributing to a better understanding of distal nephron function and of the pathophysiology of diseases in which the renal tubule is implicated, as well as to the development of new drugs.

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