A homozygous missense mutation in SCNN1A is responsible for a transient neonatal form of pseudohypoaldosteronism type 1

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1Pediatric Endocrine and Diabetes Unit, Department of the Child and Adolescent, Children’s Hospital, Geneva University Hospitals, Geneva; 2Department of Pharmacology and Toxicology, University of Lausanne, Lausanne, Switzerland. 3Institut National de la Sante et de la Recherche Medicale, U970, Paris Cardiovascular Research Center - PARCC. 4Université Paris Descartes; 5Assistance Publique-Hôpitaux de Paris, Hôpital Européen Georges Pompidou, Paris, France; and 6Pediatric Nephrology Unit, Department of the Child and Adolescent, Children’s Hospital, Geneva University Hospitals, Geneva, Switzerland

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Dirlewanger M, Huser D, Zennaro MC, Girardin E, Schild L, Schwitzgebel VM. A homozygous missense mutation in SCNN1A is responsible for a transient neonatal form of pseudohypoaldosteronism type 1. Am J Physiol Endocrinol Metab 301: E467–E473, 2011. First published June 7, 2011; doi:10.1152/ajpendo.00066.2011.—Pseudohypoaldosteronism type 1 (PHA1) is a monogenic disorder of mineralocorticoid resistance characterized by salt wasting, hyperkalemia, high aldosterone levels, and failure to thrive. An autosomal recessive form (AR-PHA1) is caused by mutations in the epithelial sodium channel ENaC with usually severe and persisting multiorgan symptoms. The autosomal dominant form of PHA1 (AD-PHA1) is due to mutations in the mineralocorticoid receptor causing milder and transient symptoms restricted to the kidney. We identified a homozygous missense mutation in the SCNN1A gene (c.727T>C/p.Ser243Pro), encoding α-subunit of ENaC (α-ENaC) in a prematurely born boy with a severe salt-losing syndrome. The patient improved rapidly under treatment, and dietary salt supplementation could be stopped after 6 mo. Interestingly, the patient’s sibling born at term and harboring the same homozygous Ser243Pro mutation showed no symptom of salt-losing nephropathy. In vitro expression of the α Ser243Pro ENaC mutant revealed a slight but significant decrease in ENaC activity that is exacerbated in the presence of high Na+ load. Our study provides the first evidence that ENaC activity is critical for the maintenance of salt balance in the immature kidney of preterm babies. Together with previous studies, it shows that, when the kidney is fully mature, the severity of the symptoms of AR-PHA1 is related to the degree of the ENaC loss of function. Finally, this study identifies a novel functional domain in the extracellular loop of ENaC.

hyponatremia; hyperkalemia; mineralocorticoid resistance; aldosterone; salt wasting

PSEUDOHYPOALDOSTERONISM TYPE 1 (PHA1) is a rare disease characterized by a resistance to mineralocorticoids that presents in the neonatal period or early infancy with a salt-wasting nephropathy, dehydration, hyperkalemia, and failure to thrive. Two major clinical forms of PHA1 have been described (17). The autosomal dominant renal form of PHA1 (AD-PHA1) involves loss-of-function mutations of the mineralocorticoid receptor (MR), encoded by the NR3C2 gene located on chromosome 4q31.1 (38, 39). More than 40 mutations have been described in both sporadic and familial cases (7, 16, 26, 36). This form of mineralocorticoid resistance is restricted to the kidney and shows moderate clinical features, with renal symptoms typically improving with age. The autosomal recessive form of PHA1 (AR-PHA1) is caused by loss-of-function mutations in the genes encoding the epithelial Na channel (ENaC) α-, β-, and γ-subunits. Symptoms are usually multisystemic, including a severe salt-losing nephropathy causing dehydration with hyperkalemia and recurrent episodes of pulmonary infection, congestion, coughing, and wheezing (21, 32); a cutaneous phenotype with eczematous rash of the skin has been reported (23, 34). The symptoms are usually persistent and more severe than in AD-PHA1. Recently, clinical improvement with age has been reported in patients with AR-PHA1 (2, 18, 28).

In the kidney, the MR is expressed exclusively in the aldosterone-sensitive distal nephron (ASDN), which includes the connecting tubule and the collecting duct (12). The ASDN is the site involved in the fine regulation of Na+ absorption and K+ secretion to balance the daily intake of these electrolytes. After binding aldosterone the MR undergoes a conformational change, and the ligand-receptor complex is translocated to the nucleus, allowing transcription of aldosterone-induced genes, including ENaC and various signaling factors (4–6, 15, 16, 27, 37–39).

ENaC colocalizes with the MR and is expressed in the ASDN of the kidney but also in other tight epithelia such as the colon, the respiratory tract, and salivary glands. In the kidney, ENaC is essential for the maintenance of the extracellular fluid and blood pressure and also plays a crucial role in K+ homeostasis. In the distal lung airways, ENaC regulates fluid absorption at the air-liquid interface, thereby determining the rate of mucociliary transport (11).

ENaC is a heteromeric protein composed of homologous subunits and provides an electrogenic sodium transport pathway from the lumen into the cell (20). The α-subunit is required for ENaC function, whereas the β- and γ-ENaC are important for maximal channel activity. The reabsorbed Na+ is then transported out of the cell into the interstitium by the Na+/K+ ATPase pump located on the basolateral membrane. In the ASDN, ENaC is the rate-limiting step in Na+ reabsorption. The electrogenic ENaC-mediated Na+ absorption in the ASDN provides the necessary driving force for K+ secretion in this part of the nephron.

In the present study, we describe a novel Ser243 to Pro mutation in the α-ENaC subunit that is associated with a

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transient AR-PHA1 diagnosed in a preterm patient with a severe salt-losing nephropathy. Our observations shed a new light on the role of ENaC in the maturing kidney and on the genotype-phenotype relations found in AR-PHA1.

METHODS.

Subjects. We studied a family presenting with an autosomal recessive form of PHA1 and sequenced the MR and ENaC genes. The parents were Sri Lankan first-degree cousins in good general health. The older child presented with clinical and laboratory characteristics of PHA1 at 16 days of age and was the index case. Informed consent was obtained from all of the family members.

Biochemical assays. Aldosterone concentrations were measured in the serum by direct radioimmunoassay with a Diagnostic Products kit (ISO 15189). The plasma renin activity (PRA) (ng·ml⁻¹·h⁻¹) was measured by direct radioimmunoassay after an incubation period at 37°C (INC Biomedical). Sweat tests and concentrations of Cl⁻, Na⁺, and K⁺ were measured according to the cystic fibrosis foundation consensus report: normal values of Cl⁻ ≤35 mmol/l and Na⁺ ≤60 mmol/l (14).

Genomic DNA isolation and sequencing. Genomic DNA was extracted from peripheral blood leukocytes by salt extraction, as described previously (26). All coding exons and the intron-exon flanking regions of the NR3C2 gene, coding for the MR, as well as the SCNN1A, SCNN1B, and SCNN1G genes were amplified with 13 pairs of primers (see Supplemental Table S1; Supplemental Material for this article is available online at the AJP-Endocrinology and Metabolism web site). Direct sequencing of PCR products was then performed by the ABI Prism Big Dye Terminator version 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) on an ABI Prism 3700 DNA Analyzer. The identified mutation was confirmed on a second PCR product and on a second DNA sample.

Site-directed mutagenesis, RNA in vitro transcription, and expression in Xenopus laevis oocytes. The human α-, β-, and γ-ENaC subunits were cloned in the pBSK vector and linearized by NotI, and capped cRNA was in vitro synthesized by SP6 polymerase. α-ENaC Ser243 to proline mutant (α-S243P) and Ser243 to cysteine (α-S243C) were obtained by site-directed mutagenesis using Stratagene’s Quick-Change protocol. Healthy stage V and VI Xenopus laevis oocytes were pressure-injected with 10 ng of human ENaC cRNA (with equal amounts of α-, β-, and γ-subunits). Oocytes were kept at 19°C in a sodium-free solution comprising 120 mM of NaCl, 0.82 MgSO₄, 0.41 CaCl₂, 0.33 Ca(NO₃)₂, 80 N-methyl-d-glucamine (NMDG), 2 KCl, and 5 HEPES or a high Na⁺ incubation solution containing (in mM) 85 NaCl, 1 KCl, 2.4 NaHCO₃, 0.82 MgSO₄, 0.41 CaCl₂, 0.33 Ca(NO₃)₂, 10 HEPES, and 4.08 NaOH.

Electrophysiology. Electrophysiological measurements were made using the standard two-electrode voltage clamp technique, using a Dagan TEV voltage clamp amplifier (Dagan, Minneapolis, MN), the Digidata 1322 digitizer, and the PCclamp 9 data acquisition and analysis package (Axon Instruments, Molecular Devices, Sunnyvale, CA). The two electrodes contained a 1 M KCl solution. All electrophysiological experiments were performed at room temperature (22°C). The holding potential was −100 mV. The composition of the perfusion solution was (in mM) 120 NaCl, 2.5 KCl, 1.8 CaCl₂·2H₂O, and 10 HEPES-H⁺ for the Na⁺ solution; NaCl was replaced by 120 mM NMDG-HEPES or KCl for the perfusion solutions devoid of Na⁺. To determine amiloride-sensitive currents, we added amiloride (Sigma) in a separated fraction of each test solution at a final concentration of 10 μM. Inward Na⁺ current was generated by switching solution without Na⁺ (NMDG or KCl) to perfusion solution containing 120 mM of Na⁺. In the experiment with proteases, the oocytes were exposed to 5 μg/ml trypsin (Sigma-Aldrich Chemie) in the 120 mM NaCl perfusion solution.

Biotinylation. For surface biotinylation 16–30 h after cRNA injection, oocytes were incubated in 1 ml of biotinylation buffer containing 1 mg/ml NHS-SS-Biotin (Sigma-Aldrich Chemie) or MTSEA-biotin (N-biotinylmaminomethyl methanethiosulfonate; Toronto Research Chemicals) for 15 min at 4°C. Oocytes were washed twice and then incubated 5 min at 4°C with 2 ml of the high Na⁺ incubation solution. Thirty oocytes per condition was lysed with 20 μl lysis buffer/oocyte. Oocytes were vortexed and centrifuged for 10 min at 12,000 rpm (4°C). The intermediate phase was withdrawn, and a sample of each lysate was kept as a control of “total lysate fraction.” Lysates were then incubated overnight with 40 μl of streptavidin beads (ImmunoSorp Immobilized Streptavidin Gel; Perbio) at 4°C.

Statistical analyses. Student’s t-test was used for statistical analysis. Data are expressed as means ± SE.

RESULTS.

Clinical course. The index case issuing from consanguineous parents was born prematurely at 32 wk of gestation by vaginal delivery following premature rupture of the membranes. The patient’s birth weight was 1,645 g (between the 10th and 50th percentile, growth curve adjusted for ethnicity and age) (24). The newborn developed hypotonia and feeding problems, and no respiratory distress was noted. On day 16 of life, severe hyponatraemia associated with hyperkalemia and mild metabolic acidosis (pH 7.30) was discovered. Plasma renin activity was elevated, as was the plasma aldosterone level (Table 1). Fractional sodium excretion (FENa) was increased, and the urinary Na⁺/K⁺ ratio was 7.6 (normal value <2), confirming salt wasting (Table 1) (18, 25). These results were consistent with the diagnosis of PHA1. Treatment with cation exchange resin (0.5 g·kg⁻¹·day⁻¹) and NaCl (6 mmol·kg⁻¹·day⁻¹) supplementation was introduced. At 7 wk after birth, evolution of the FENa and serum Na⁺ values normalized under treatment, reaching values comparable with the patient’s sibling born at term (Fig. 1). Salt supplementation could be stopped at 6 mo of life without subsequent salt-losing episodes.

Table 1. Clinical characteristics of the family members with PHA1

<table>
<thead>
<tr>
<th>ID</th>
<th>Age</th>
<th>Genotype</th>
<th>Aldosterone, ng/dl</th>
<th>PRA, ng·ml⁻¹·h⁻¹</th>
<th>Na⁺, mmol/l</th>
<th>K⁺, mmol/l</th>
<th>FENa, %</th>
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</thead>
<tbody>
<tr>
<td>Index patient</td>
<td>2 wk</td>
<td>−/−</td>
<td>2088</td>
<td>&gt;500</td>
<td>106</td>
<td>7.9</td>
<td>5.52</td>
</tr>
<tr>
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<td>6 mo</td>
<td>−/−</td>
<td>148</td>
<td>2.33</td>
<td>139</td>
<td>4.5</td>
<td>0.11</td>
</tr>
<tr>
<td>Brother</td>
<td>2 wk</td>
<td>−/−</td>
<td>1058</td>
<td>220</td>
<td>136</td>
<td>5.9</td>
<td>0.12</td>
</tr>
<tr>
<td>Brother</td>
<td>4 mo</td>
<td>−/−</td>
<td>148</td>
<td>140</td>
<td>4.7</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Mother</td>
<td>36 yr</td>
<td>±</td>
<td>5.0</td>
<td>1.00</td>
<td>139</td>
<td>4.1</td>
<td>NA</td>
</tr>
<tr>
<td>Father</td>
<td>40 yr</td>
<td>±</td>
<td>14.0</td>
<td>1.93</td>
<td>140</td>
<td>4.2</td>
<td>NA</td>
</tr>
</tbody>
</table>

PHA1, pseudohypoaldosteronism type 1; PRA, plasma renin activity; FENa, fractional sodium excretion. −/− homozygous; ± heterozygous for Ser243Pro (S243P) mutation. Normal values for aldosterone 19–141 (premature infant), 5–90 (full-term infant), and 4–30 ng/dl (adult). Normal values for PRA 11–167 (premature infant), 2.35–37 (full-term infant), and 0.82–3.68 ng·ml⁻¹·h⁻¹ (adult). Na⁺ 131–145 mmol/l (newborn). K⁺ 3.6–5.9 mmol/l (newborn). FENa <2%.
ENaC mutation in a transient form of pseudohypoaldosteronism

The second child of the same parents was born at term also by vaginal delivery and did not develop any electrolyte abnormality, and urinary Na⁺/K⁺ ratio, as well as the FENa, was normal (0.36; Table 1). However, the aldosterone level and PRA at 2 wk of age was above the normal range for age (Table 1). Sweat tests were performed in both brothers. The chloride concentration was normal for both children [38 and 16 mmol/l, respectively, (n = 39 mmol/l)], excluding the diagnosis of cystic fibrosis. Sweat sodium concentrations were normal [53 and 27 mmol/l (n = 9–72 mmol/l)]. Both parents showed normal values of aldosterone and PRA without any electrolyte disturbances (Table 1).

Genetic analysis. Given the exclusive renal symptoms of index case, we initially hypothesized an AD-PHA1 and performed direct sequencing of the NR3C2 coding exons and the intronic exon-flanking regions for confirmation. No mutation was found in the NR3C2 amplified from the index case. Subsequent analysis of the genes coding for the subunits of the epithelial sodium channel ENaC revealed a homozygous c.727T→C mutation in exon 4 of the SCNN1A gene, substituting a proline (Pro) for serine (Ser) at position 243 (Ser243Pro) in the α-ENaC subunit. No other mutations were detected in the SCNN1B and SCNN1G genes coding for the β- and γ-ENaC subunits, respectively. Both the index case and the patient’s younger asymptomatic brother were homozygous carriers of the same Ser243Pro mutation (α-S243P), whereas the mother and the father were heterozygous. The Ser243 residue lies in the large extracellular domain of ENaC within the second cysteine-rich domain and is highly conserved among the α-, β-, and γ-ENaC subunits of different species from amphibian to mammalian (Supplemental Fig. S1).

Functional analysis of the mutant α-ENaC. The ENaC α-S243P mutant was expressed in Xenopus oocytes for functional analysis. Representative tracings of the Na⁺ current mediated by ENaC wild type (WT) and ENaC α-S243P mutant are shown in Fig. 2, A and B. The rapid substitution of an external solution devoid of Na⁺ ions to a solution containing 120 mM Na⁺ generated a robust inward current characterized by a large peak current followed by a current relaxation to a steady-state current after 10 s. The steady-state current was inhibited by the addition of 10 μM amiloride to the bathing solution. The α-S243P mutant exhibits an amiloride-sensitive, inward Na⁺ current that is qualitatively similar to WT, but quantitatively the current magnitude is lower. This decrease in the steady-state current magnitude is summarized in Fig. 2, C and D. ENaC current was measured in oocytes expressing either ENaC WT or the α-S243P mutant after overnight incubation of the oocytes in a high or low Na⁺-containing medium. Since ENaC is constitutively open when expressed at the cell surface, extracellular Na⁺ equilibrates with the intracellular Na⁺ and thus determines both the extra- and intracellular Na⁺ loads (1). Under both conditions, the S243P mutant exhibits a lower amiloride-sensitive current (Fig. 2C). The overnight incubation in a high Na⁺ medium (85 mM NaCl) of oocytes expressing ENaC WT or the S243P mutant results in a significant decrease in ENaC current compared with oocytes preincubated overnight in a low Na⁺ medium (10 mM NaCl). This phenomenon has been described extensively and is called feedback inhibition of the channel in response to an increase in intracellular Na⁺ load (1). The feedback inhibition of ENaC serves to prevent excessive accumulation of intracellular Na⁺ ions that could be toxic for the cell. Compared with WT ENaC, the reduction in the amiloride-sensitive Na⁺ current expressed by the α-S243P mutant was more pronounced (80%) after overnight incubation in the high Na⁺-containing medium than in the low Na⁺ medium (30% decrease) (Fig. 2, C and D). These experiments show that the α-S243P mutation is associated with a partial but significant loss of function. In addition, this loss of function is exacerbated under conditions favoring a high intracellular Na⁺ load as if the α-S243P mutation makes the channel hypersensitive to feedback inhibition in the presence of a high Na⁺ load.

The ENaC current relaxation from the maximal peak current to its steady state is a fast adaptive process dependent on the permeating Na⁺ ions, termed ENaC self-inhibition (8). We asked whether this physiological inhibitory response to extra-
cellular Na⁺ ions was modified in the α-S243P mutant. Self-inhibition was defined as the ratio of the maximal peak current over the steady-state current measured at 20 s (Iss/Ipeak); the higher the ratio, the lower the ENaC self-inhibition. The data in Fig. 3 show that for ENaC WT and α-S243P mutant, the self-inhibition is independent of the incubation conditions in high or low Na⁺ medium. The ratio Iss/Ipeak for the α-S243P mutant was significantly decreased for the S243P mutant compared with ENaC WT, indicating a slightly stronger apparent self-inhibition for the channel mutant. The kinetic parameters of the self-inhibition of the S243P mutant, obtained from the exponential fit of the Na⁺ current decrease, were similar for both the ENaC WT and mutant channel (data not shown). Thus the apparent stronger feedback inhibition determined by the Iss/Ipeak ratio is likely due to a lower steady-state current for the α-S243P mutant, as shown in Fig. 2.

We have verified that the reduction of ENaC-mediated current for the α-S243P mutant was not due to a lower expression of ENaC at the cell surface. Mutant and WT channels at the cell surface were labeled with biotin and affinity purined on streptavidin beads. Western blots in Fig. 4 using an anti-α-ENaC antibody show the intracellular (unbound fraction to streptavidin beads) and surface expression (bound fraction to streptavidin beads) of the ENaC mutant compared with the WT. Both intracellular and surface expression of the ENaC WT or α-S243P mutant were comparable, and therefore, changes in the expression of the α-S243P mutant at the protein level cannot account for the observed decrease in amiloride-sensitive current. In addition, we also tested the possibility that the α-S243P mutation may change channel affinity for amiloride. Both ENaC WT and S243P mutant have comparable affinities for amiloride with an IC₅₀ of 0.29 (± 0.08; n = 12) and 0.15 μM (± 0.04; n = 12), respectively.

Fig. 2. Epithelial Na channel (ENaC)-mediated Na⁺ currents in oocytes expressing ENaC wild type (WT) and ENaC α-S243P mutant. A and B: representative recordings of amiloride-sensitive Na⁺ current in oocytes expressing ENaC WT αβγ-WT and ENaC α-S243Pβγ mutant. C: absolute amiloride-sensitive steady-state Na⁺ currents measured in oocytes incubated for 24 h (see METHODS) in a low Na⁺-containing solution (closed bars; n = 12 experiments) and in a high Na⁺ solution (open bars). **P < 0.01; ***P < 0.001.

Fig. 3. ENaC self-inhibition of the WT channel and the α-S243P mutant. Channel self-inhibition was defined as the ratio of the steady-state current (Iss) measured after 20 s over the peak current (Ipeak) (see Fig. 2, A and B). Closed and open bars, low and high Na⁺ incubation conditions, respectively. Iss/Ipeak ratio for the α-S243Pβγ and the WT was 0.57 ± 0.04 and 0.70 ± 0.03, respectively (Student’s t-test; P < 0.05, n = 12), and 0.51 ± 0.04 vs. 0.65 ± 0.04 (Student’s t-test; P < 0.01, n = 8).

What is the mechanism underlying the partial loss of ENaC function due to the α-S243P mutation? Several mechanisms regulate ENaC at the cell surface. One mechanism associated with the activation of ENaC at the cell surface involves a proteolytic cleavage of the α- and the γ-subunit of the ENaC

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complex by serine proteases at specific sites in the extracellular domain (29); this channel activation can be reproduced using low concentrations of external trypsin in Xenopus oocytes (9). Although α-S243 is not part of a consensus target sequence for serine proteases, we verified that the loss of function of the α-S243P mutant channel was not due to a resistance to cleavage and channel activation by serine proteases. Experiments in Fig. 6 confirm the partial loss of function of the α-S243P mutant after overnight incubation in a low or high Na⁺ medium. In the presence of trypsin, both the WT and the mutant show an approximately twofold increase in channel activity; the reduced activity of the α-S243P still remained significant compared with the WT, even in the presence of trypsin. These experiments indicate that the α-S243P mutant retains its sensitivity to activation by extracellular trypsin, but the loss of function of the channel mutant cannot be overcome by trypsin; thus the mechanism underlying the channel loss of activity is a more general mechanism that is independent of the channel activation by proteolytic cleavage.

The substitution of the α-Ser²⁴³ by a proline is expected to markedly change the architecture of the protein at this specific site, since Pro is often found in bends of folded proteins. We could observe that a more conserved substitution of the Ser²⁴³ by cysteine was without effect on ENaC activity. Actually, after overnight incubation in a high Na⁺ medium, α-S243Cβγ, did not display a steady-state current that was significantly different from the WT (15.7 μA ± 3.14; n = 24, P = 0.08). Thus it is likely that the S243P mutation in the α-ENaC introduces important steric changes in an extracellular domain that is important for ENaC activity and its modulation.

**DISCUSSION**

In this study, we have identified in a patient with a transient form of AR-PHA1 a novel homozygous mutation in the *SCNN1A* gene, leading to a Ser²⁴³ to Pro substitution of the α-ENaC subunit. This patient was born prematurely at week 32 and presented renal symptoms only, characterized by a severe salt-wasting nephropathy with extremely high levels of aldosterone. Within 2 wk of treatment with salt supplementation and cation exchange resin, clinical symptoms rapidly improved, and serum and urinary Na⁺ values normalized. Blood analyses performed at 6 mo of age still revealed an elevated aldosterone level but without electrolyte disturbances. The patient’s younger brother born at term with the same homozygous α-S243P mutation did not show any renal salt-wasting phenotype, only an elevated plasma aldosterone.

In premature infants the urinary loss of sodium exceeds the daily intake, leading to a negative Na⁺ balance in most infants less than 28 wk of gestational age (GA) (3, 10). A positive balance is achieved only after 32 wk of GA, which is due to continuing kidney maturation responsible for a decrease in renal fractional excretion of sodium. The cellular mechanisms for the limited capacity of the kidney to retain Na⁺ before term have not been completely elucidated.

Microperfusion of cortical collecting ducts isolated from 1-wk-old rabbits with immature kidneys showed no significant Na⁺ transport, and no conducting ENaC channels could be observed by patch-clamp technique (30, 31). To the extent that the immature kidney of newborn rabbits is a representative model of the premature human kidney, the paucity of active ENaC channels may certainly contribute to the reduced capacity of the immature kidney to reabsorb Na⁺ ions. In addition, a relative insensitivity of the immature kidney to aldosterone has been demonstrated in the newborn rat and rabbit, suggesting that the plasma mineralocorticoids play a limited role in regulating renal distal Na⁺ absorption in the early postnatal life (35).

The transition from the fetal to the neonatal life is characterized by a dramatic decrease in the urinary Na⁺ excretion. Clearance studies using diuretics have suggested that nephron segments downstream of the proximal tubule are responsible...
for the sharp increase in Na\(^+\) absorption during the early postnatal life (33). Patch-clamp studies on rabbit neonates have shown that the product of the number of ENaC channel times the channel open probability increases 30-fold between the 1st and the 2nd wk after birth (31).

The index case shows a urinary Na\(^+\) excretion, as measured by the UNa\(^+\)/K\(^+\) ratio, more than three times higher than expected for a preterm infant of 32 wk of GA (3). The identification of the \(\alpha\)-S243P ENaC loss of function mutation in this patient is the most likely explanation for the patient’s abnormally high UNa\(^+\)/K\(^+\) and the high plasma aldosterone level. The evidence \(\alpha\)-S243P mutation results in a channel loss of function confirms the diagnosis of AR-PHA1. Our observation represents the first direct evidence that ENaC plays a crucial role for achieving a Na\(^+\) balance starting a 32 wk of GA, since a slight decrease in ENaC activity due to the \(\alpha\)-S243P mutation has dramatic consequences on distal Na\(^+\) absorption and can precipitate a severe salt-wasting nephropathy in a premature baby.

The rapid clinical improvement of our patient during the first weeks of life is likely due to kidney maturation associated with an increase in the expression of ENaC channels as well as other Na\(^+\) transporters in or upstream of the distal nephron. This is supported by the fact that the patient’s younger brother, carrying the same genotype but born at term, never developed the renal symptoms but had a slightly elevated plasma aldosterone level. This further indicates that limited loss of function of the \(\alpha\)-S243P ENaC mutation is not sufficient to precipitate an AR-PHA1 when the kidney maturation is nearly completed at term. However, we cannot exclude potential epigenetic changes contributing to the different phenotypes, as sometimes observed in monozygotic twins.

The parents of the index case, heterozygous for the mutation, do not show any sign of a Na\(^+\)-losing nephropathy despite an estimated 40% reduction in ENaC activity. Similarly, the partial loss of ENaC function due to the mutation \(\alpha\)-S243P has no effects on airway fluid clearance or on the electrolyte composition of the sweat. These observations point out the importance of compensatory mechanisms in the kidneys, lungs, and other organs expressing ENaC to overcome a limited reduction of its activity.

Clinical improvements of AR-PHA1 have already been reported, but in most cases patients exhibit salt-losing episodes if they are not supplemented with dietary salt (13, 18, 28). Clinical improvement is usually associated with missense mutations or mutations in the \(\beta\)- or \(\gamma\)-subunits, resulting in only partial but not in complete loss of ENaC function. The slight reduction in ENaC activity of the \(\alpha\)-S243P mutant fully supports the notion that the clinical evolution of the AR-PHA1 is related to the degree of ENaC loss of function caused by the genetic mutations. A partial loss of function of ENaC in the ASDN is likely to be compensated by an increased Na\(^+\) absorption in the upstream nephron segments. The late distal nephron also responds to elevated plasma levels of aldosterone by increasing the expression of the thiazide-sensitive NaCl cotransporter (NCC) (22). Recently, an increased level of NCC protein in the urine of a patient with AR-PHA1 was reported and supports the hypothesis of a compensatory Na\(^+\) absorption in the distal nephron (2, 19). This upregulation of the NCC likely represents a compensatory mechanism to reabsorb Na\(^+\) and to maintain Na\(^+\) balance in the mature kidney but not in an immature kidney at the GA of 32 wk.

In the premature kidney, an elevated delivery of Na\(^+\) is expected in the distal part of the nephron where ENaC is expressed and can result from upstream immature functional Na\(^+\) transporters; we tried to experimentally reproduce this situation with oocytes expressing ENaC by overnight incubation in a high Na\(^+\) medium, leading to intracellular Na\(^+\) load. Interestingly, it was under such a high Na\(^+\) load that we observed the most important loss of ENaC function. We still do not understand the molecular mechanism underlying this effect, but it may also contribute to the salt-losing episode after birth and the rapid clinical improvement of our preterm patient. Indeed, as the Na\(^+\) transporters mature in the nephron segments upstream of the ASDN, the distal Na\(^+\) delivery decreases, and under these conditions we can expect from our data that the degree of ENaC loss of function is reduced.

Finally, the S243P mutation identifies a novel functional domain in the extracellular loop of the channel that may link ENaC activity to changes in chronic extracellular Na\(^+\) load. The S243 residue is located in the extracellular loop of the channel that may link ENaC activity to changes in chronic extracellular Na\(^+\) load. The S243P mutation results in a channel loss of function mutation in the extracellular loop of ENaC that is important for channel regulation.

**Fig. 6. ENaC channel activation by trypsin.** The addition of trypsin (5 \(\mu\)g/ml) in the external medium (open bars) significantly increased the current of the mutant \(\alpha\)-S243P\(\beta\gamma\) and the WT 2.6- and 1.9-fold, respectively, after incubation in a low Na\(^+\) medium and 3- and 2.2-fold, respectively, after incubation in a high Na\(^+\) medium. The number of oocytes ranged from 17 to 26. ***P < 0.001; **P < 0.01.
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No conflicts of interest are reported by the authors.


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