Renin–aldosterone response, urinary Na/K ratio and growth in pseudohypoaldosteronism patients with mutations in epithelial sodium channel (ENaC) subunit genes

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ARTICLE INFO

ABSTRACT

Multi-system pseudohypoaldosteronism (PHA) is a rare syndrome of aldosterone unresponsiveness characterized by symptoms of severe salt-losing caused by mutations in one of the genes that encode α, β or γ subunit of epithelial sodium channels (ENaC). We examined long-term changes in the renin–aldosterone response in patients with different mutations. Four PHA patients were followed-up for 7–22 years. Patient A with a heterozygous Gly327Cys missense mutation in αENaC is a mild case and patients B, C and D are severe cases. Two additional patients with renal PHA served as controls. In patient A, serum aldosterone and plasma renin activity (PRA) decreased with age, PRA reaching near normal values at age 11. In contrast, patients B–D showed a positive correlation between age and aldosterone ($r > 0.86$ for all). In patient B with Arg508 stop mutation, aldosterone reached 166 nmol/L at age 19 (>300-fold higher than normal). Urinary Na/K ratios normalized gradually with age in all patients. Growth curves of the patients were reflective of the severity of PHA and compliance with salt therapy. Functional expression studies in oocytes showed that ENaC with αGly327Cys mutation, as observed in patient A, showed nearly 40% activity of the wild type ENaC. In contrast, stop mutation as in patient B reduces ENaC activity to less than 5% of the normal. Our results demonstrate distinct genotype–phenotype relationships in multi-system PHA patients. The degree of ENaC function impairment affects differently the renin–aldosterone system and urinary Na/K ratios. The differences observed are age-dependent and PHA form specific.

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1. Introduction

Pseudohypoaldosteronism (PHA) type I is a rare syndrome of aldosterone unresponsiveness that is characterized by symptoms of severe salt-losing including hyperkalemia, hyponatremia, hyperaldosteronism and hyperreninemia [1,2]. Analyses of a series of patients revealed that PHA includes two distinct entities with different clinical, biochemical and hereditary characteristics [3]. The severe form of multi-system PHA is caused by loss-of-function mutations in one of the three genes that encode α, β or γ subunit of epithelial sodium channels (ENaC) [4–11]. This form results in severe salt-losing from all aldosterone responsive target organs, such as kidney, colon, sweat and salivary glands. The patients also suffer from recurrent lower respiratory tract infections [7,12]. Many cases of the second milder form of PHA (renal PHA) have been shown to be a result of heterozygous mutations in the gene encoding the mineralocorticoid receptor [13,14]. In this form, salt-loss is limited to distal renal tubules.

Most of the mutations in ENaC subunit genes responsible for multi-system PHA are nonsense, single base deletion or insertion, or splice-site mutations that result in the formation of truncated subunits [11]. There are only three reported cases of missense mutations [4,11,15]. The clinical course of multi-system PHA has been well established during infancy and early childhood. However, there is no information on the long-term consequences of the different types of ENaC mutations and on the function of the renin–aldosterone system and growth and pubertal development

of PHA patients throughout adolescence and early adulthood. Two previous studies that have examined multi-system PHA patients up to 7–9 years of age have not included long-term evaluation of the PRA, aldosterone or urinary Na/K ratio [8,15]. Bistritzer et al. reported PRA and aldosterone levels in a pair of twins with multi-system PHA up to 6 years of age [16].

In this study we present the results of long-term follow-up of four multi-system PHA patients with different types of ENaC gene mutations up to young adulthood. The biochemical and developmental parameters in these PHA patients reveal major changes in the renin–aldosterone axis function and urinary Na/K ratios that depend on the genotype. Similarly, the growth parameters, pubertal development and clinical course also showed genotype related differences. This study presents for the first time extensive data on the long-term response of the renin–aldosterone system in humans with impaired or absent ENaC function.

2. Materials and methods

2.1. Patients

We examined four multi-system PHA patients (A, B, C and D) who were followed-up for seven to 22 years of age. Patient A is a compound heterozygote with one missense (Gly327Cys) and one frameshift (His450fs) mutation in SCNN1A gene encoding αENaC [11]. Patient B carries a homozygous SCNN1A Arg508 stop mutation [4]. Sequencing of the coding regions of all three ENaC genes, including the promoter regions, did not reveal a mutation for patient C (previously unpublished results). Patient D carries a splice-site mutation (1669 + 1G → A) in intron 12 of SCNN1B gene encoding βENaC [11].

All four cases exhibited symptoms of severe salt-losing including dehydration, acidosis, hyponatremia and hyperkalemia during the first 10 days of life. Sweat and salivary Na and chloride concentrations were increased in all, consistent with multi-system PHA classification [3]. All the patients were on oral NaCl therapy at a dose ranging from 8 to 20 g/NaCl per day. Patient D receives in addition Kayexalate.

We also followed-up two patients with renal PHA (Patients E and F) for 30 and 15 years who served as controls. They are members of a large kindred with 16 affected members over 4 generations. The genetic and initial endocrine evaluation of patient E who was the propositus, was previously reported [3,17].

2.2. Biochemical assays

Aldosterone concentrations were measured by radioimmunoassay (DPC Coat-a-Count aldosterone kit, Diagnostic Products Corporation, Los Angeles, CA). Plasma Renin activity (PRA) was measured using Gammacoat PRA kit (Diasorin, Stillwater, MN). Growth parameters including height, weight, growth velocity and the respective SDS values were analyzed by Growth Analyzer software (Dutch Growth foundation-Rotterdam, Netherlands). Height-for-age growth-curves were based on the 1990 British cross-sectional height standards.

2.3. cDNA cloning, sequencing and ENaC activity assays

To measure the activity of ENaC we generated three cDNAs encoding for the α, β and γ subunits of human ENaC that were subcloned in plasmid pGEM-HJ. An αENaC cDNA clone with the p.Gly327Cys missense mutation was generated by site-directed-mutagenesis of the normal αENaC cDNA. Both the wild types and the mutated form were completely sequenced using an ABI 310 Genetic Analyzer to verify the sequences. In vitro transcriptions of the cDNAs were carried out using T7-RNA polymerase. The cRNAs (3 ng for each subunit) were micro-injected into immature stage V–VI Xenopus oocytes that were dissociated with 0.3 mg/mL type 1A collagenase [18]. The oocytes were incubated at 18 °C in ND-96 medium (in mM: 96 NaCl, 2 KCl, 1 CaCl$_2$, 1 MgCl$_2$, and 5 HEPES, pH 7.4) containing 2.5 mM sodium pyruvate, 50 μg/mL gentamicin and 10 μM amiloride. ENaC dependent amiloride-sensitive whole-cell inward Na$^+$ current was measured 2–3 days after cRNA injection using the two-electrode voltage-clamp method while oocytes were clamped at −80 mV and continuously perfused with ND-96 + 10 μM amiloride and ND-96 alternately at 22 °C. Current–voltage relationship were also recorded in ND-96 medium. In the experiments with Li ions, LiCl was substituted for NaCl. Data were collected and analyzed using pClamp software (Axon Instruments).

2.4. Statistical analysis

Significance of the difference between means were analyzed by t-test or analysis of variance as appropriate.

3. Results

3.1. Serum aldosterone and PRA

Serum concentrations of aldosterone, PRA and urinary Na/K ratios were determined for all patients from infancy until the age of 7–22 years. Since acute episodes of salt-wasting affect renin–aldosterone system function, we excluded aldosterone and PRA results during such episodes. Patient B received intermittent GH treatment between the ages of 7.9–9.5 and 12–14.9, because of GH deficiency. Since GH affects renin–aldosterone system [19], aldosterone and PRA values during GH treatment were also excluded from the results presented below.

In normal infants, the levels of serum aldosterone and PRA decrease during the first months of life. In multi-system PHA patients, serum aldosterone and PRA levels also show a decrease during infancy, but their levels remain much higher than normal [3]. In patient A with Gly327Cys missense mutation, both aldosterone concentration and PRA decreased with age (Fig. 1). There was a strong negative correlation between age and aldosterone values ($r = -0.91$, $P < 0.01$). The correlation between aldosterone and PRA values was also high ($r = 0.71$, $P < 0.05$).

In contrast, beyond infancy, patients B, C and D with severe multi-system PHA showed a positive correlation between age and aldosterone. In patient B with Arg508stop mutation, serum aldosterone increased continuously reaching 129–166 nmol/L, concentrations that are up to 300-fold higher than normal values (Fig. 1). Age and aldosterone values were highly correlated ($r = 0.85$, $P < 0.01$). The correlation between aldosterone and PRA values for the age range 4–20 years was $r = 0.54$ ($P < 0.05$). In patient C, aldosterone levels increased from a mean of 23.5 nmol/L, at the age eight, to 166 nmol/L, at the age of 22 years (age vs. aldosterone: $r = 0.89$, $P < 0.01$). During the last 3 years, aldosterone concentrations at ages 18.9, 20.7 and 22.2 were 165, 85 and 166 nmol/L, respectively. In patient D with a βENaC splice-site mutation, aldosterone increased from 0.5 nmol/L at 3 years of age, to 2.9 nmol/L at 6.5 years. As patient D is 7 years old, it remains to be seen whether the trend of increase in aldosterone will continue with age. The serum electrolytes of the patients during follow-up visits were generally within the normal range with the exception of patient C who occasionally presented with hyponatremia in some of her visits (e.g., Na: 129 at age 20.7).

The two renal PHA patients E and F resembled in their patterns of aldosterone and PRA, but showed drastically different results from
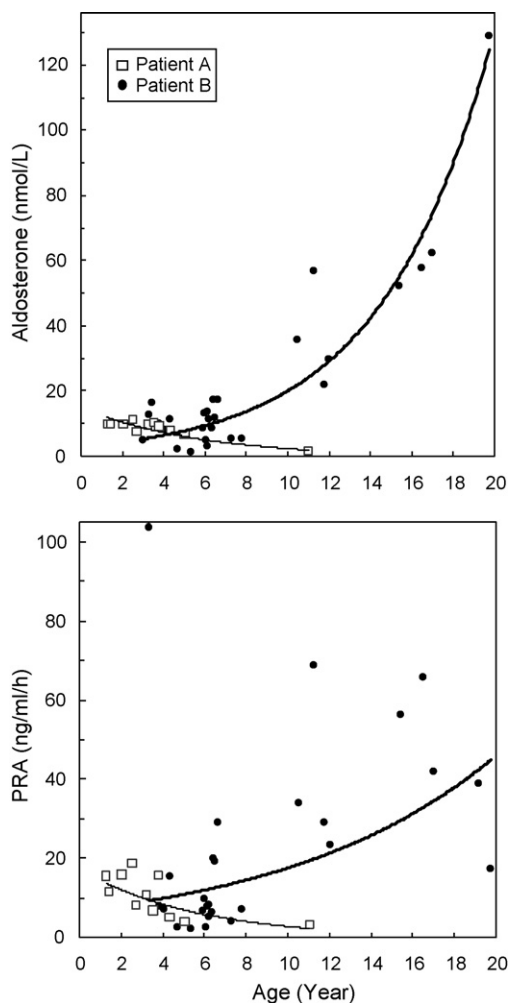
Fig. 1. Serum aldosterone and plasma renin activity (PRA) as a function of age in multi-system PHA patient A (αENaC Gly327Cys) (empty squares) and patient B (R508stop) (filled circles). In patient B the value of aldosterone at the age of 19.2 years (166.2 nmol/L) is not shown as it is off-scale, consistent with the trend of increase with age. Normal ranges: aldosterone 0.06–0.4 nmol/L; PRA <3 ng/mL h.

the multi-system cases noted above (Fig. 2). Both during infancy and throughout follow-up their aldosterone and PRA levels were much lower than those of the multi-system PHA cases. Again in contrast to the multi-system cases, their aldosterone levels remained generally stable from the age of 2–15 years (Patient E at the age of 30 shows values in the same range) but higher than normal values (Fig. 2). The PRA values show a slight but consistent tendency to decrease by age, reaching near normal values (Fig. 2).

3.2. Na/K ratio

The normal urinary Na/K ratio in children is about 2 [20]. In all multi-system PHA patients this ratio was very high during the first year of life indicating impaired aldosterone activity (Fig. 3). The Na/K ratios decreased gradually with age in all patients (Fig. 3). The age-dependent decrease in the urinary Na/K ratio was most evident for patient B for whom we have the most extensive evaluation up to 20 years of age (Fig. 3) (age vs. urinary Na/K ratio: $r = -0.69, P < 0.01$). The improvement of the ratio was mainly due to increase in urinary potassium levels.

Patient A had the lowest Na/K ratios throughout follow-up, starting already during the first year of age. The mean ratio for patient A differed significantly from the mean ratio of the severe PHA cases
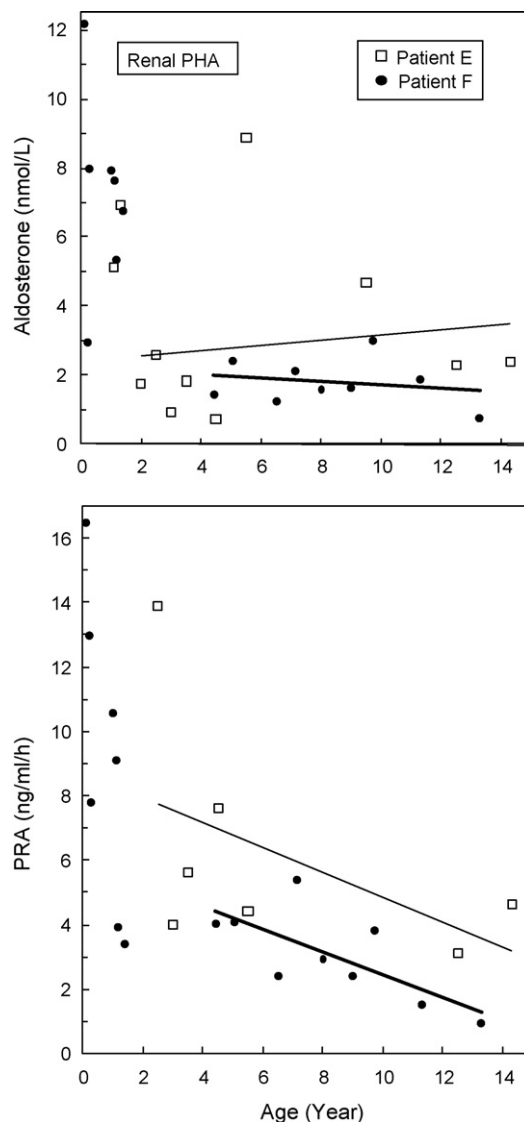

Fig. 2. Serum aldosterone and plasma renin activity (PRA) as a function of age in renal PHA patients E (empty squares) and F (filled circles).

during both infancy (9.3 vs. 55.1, $P < 0.01$) and in the 1–11 age group (4.3 vs. 14.0, $P < 0.001$). Throughout the follow-up period, sweat and salivary Na^+ and Cl^- concentrations remained persistently elevated and sweat Na/K ratio did not show significant change in all patients (data not shown).

The renal PHA cases did not show age-dependent changes in the urinary Na/K ratio beyond one month of age. The mean ± S.D. values for patients E and F were 1.6 ± 0.9 and 1.9 ± 1.2, respectively ($N = 12$ and $N = 10$), which were generally in the normal range.

3.3. Clinical course

In multi-system PHA patients, the clinical course varied both due to nature of the mutation and compliance with the recommended therapy. All the patients, except Patient A, exhibited the typical course of multi-system PHA during the first years of life, including life threatening, repeated salt-wasting episodes and frequent respiratory tract infections requiring hospitalization. With increasing age we observed an improvement in these parameters in all patients. During regular follow-up visits the blood pressure was normal in all patients.
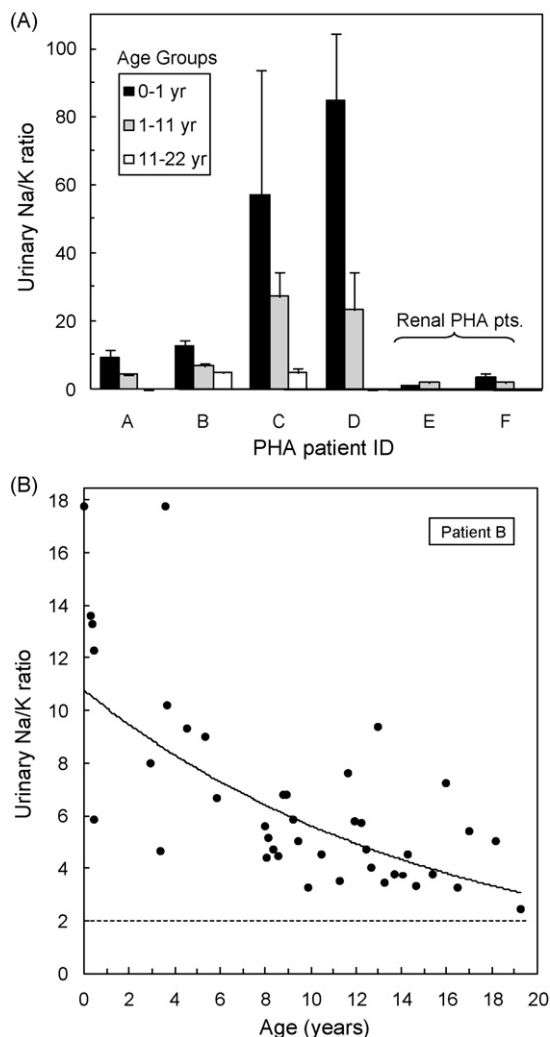
(A)

(B)

Fig. 3. (A) Age-dependent changes in urinary Na/K ratio in four multi-system PHA and two renal PHA patients. The number of determinations for patients: (A) 5 and 3; (B) 5, 18 and 18; (C) 2, 7 and 3; (D) 7 and 4; (E) 5 and 7; (F) 4 and 8, respectively, for age groups 0–1, 1–11 and 11–22. For patients A, B and D the age-group means differed significantly ($P < 0.05$). (B) Age-dependent changes in urinary Na/K ratio in multi-system PHA patient B.

In Patient A, salt-wasting episodes were very rare. His last salt-wasting episode was at the age of 9 years (serum Na 126 mmol/L; K 5.1 mmol/L). He did not suffer from recurrent lower respiratory tract infections. He requires the lowest amount of NaCl per day as compared to other patients (Table 1).

In patient B, the salt-wasting episodes and respiratory infections became less severe with age. Her last two hospitalizations for bronchopneumonia and salt-wasting episode were at the ages of 15 and 20 years, respectively. She still requires high amounts of NaCl (Table 1).

Patient C did not comply with the therapy. In her rare follow-up visits she usually presented with hyponatremia and hyperkalemia

(serum Na in the range of 125–129 mmol/L; K 7.1–7.4 mmol/L) indicating chronic salt depletion. Despite chronic salt depletion, during the last 7 years she did not have a severe salt-wasting episode or lower respiratory tract infection requiring hospitalization. During her last visit at the age of 23 her serum Na 143.5 mmol/L and K was 6.5 mmol/L.

In patient D, since the age of 4 years, salt-wasting episodes and lower respiratory infections became less frequent (1–2 hospitalizations per year). He consumes salt and Kayexalate at recommended amounts (Table 1).

3.4. Growth and pubertal development

All patients, except patient A, exhibited poor growth and short stature (Table 1 and Fig. 4). Growth curves were reflective of the severity of PHA and the degree of compliance with salt therapy. Patient A grew along the 50–75 percentiles after neonatal period (Fig. 4A).

Patient B was diagnosed with growth hormone (GH) deficiency at the age of 7.9 years [19]. The bone age before GH therapy was 4 years. On GH therapy, height SDS improved significantly from −3.55 to −2.65. The GH therapy was stopped at 9.8 years by the parents because of increased weight and muscle mass. Yet, because of gradual decrease in height SDS, GH therapy was restarted at the age of 12.1 years resulting again in improved height SDS from −3.23 before the therapy to −2.4 at the end of treatment. Therapy was stopped at 14.9 years (Fig. 4). Repeated GH test results at the age of 17 were compatible with the diagnosis of GH deficiency (peak GH responses to insulin and clonidine 4.5 and 0.6 μg/L, respectively). Her final height was 3.4 cm below her target height (−2.0 SDS). She was slightly overweight. At 13 years she had Tanner stage II breast and pubic hair. Her first menstruation was at 14.9 years. Currently she is a married woman and menstruating regularly.

Patient C exhibited poor growth and markedly delayed puberty due to poor compliance with salt therapy. At the age of 16.5 years a small uterus and ovaries were visible in abdominal ultrasound. At 20.5 years of age her serum estradiol level was undetectable (below 5 pg/mL). She had Tanner stage III breast development and Tanner stage II pubic hair and no axillary hair. She was relatively obese (Table 1). At the age of 18.7 years her bone age was 12. At 22 years of age she still did not attain final height and full puberty.

In patient D, the gastrostomy operation at the age of 1 year improved his height percentile from below third to 10 (Fig. 4A).

3.5. Activity of ENaC with Gly327Cys mutation in αENaC subunit

As noted above, the clinical profile of Patient A with missense mutation was distinctly different from the other three severe cases. Therefore, we carried out functional expression studies in oocytes to examine the activity of ENaC with the same type of Gly327Cys mutation in αENaC. As previously reported [21], the full activity of ENaC was dependent on the expression of all three subunits of ENaC (Fig. 5). Expression of αENaC cRNA with Gly327Cys mutation in combination with normal β- and γENaC, resulted in a significant reduction of ENaC dependent Na$^+$ current, as compared to the control WT ENaC (37% vs. 100%, $P < 0.02$). To examine if the muta-

Table 1
Growth parameters of the multi-system PHA patients at their last visit

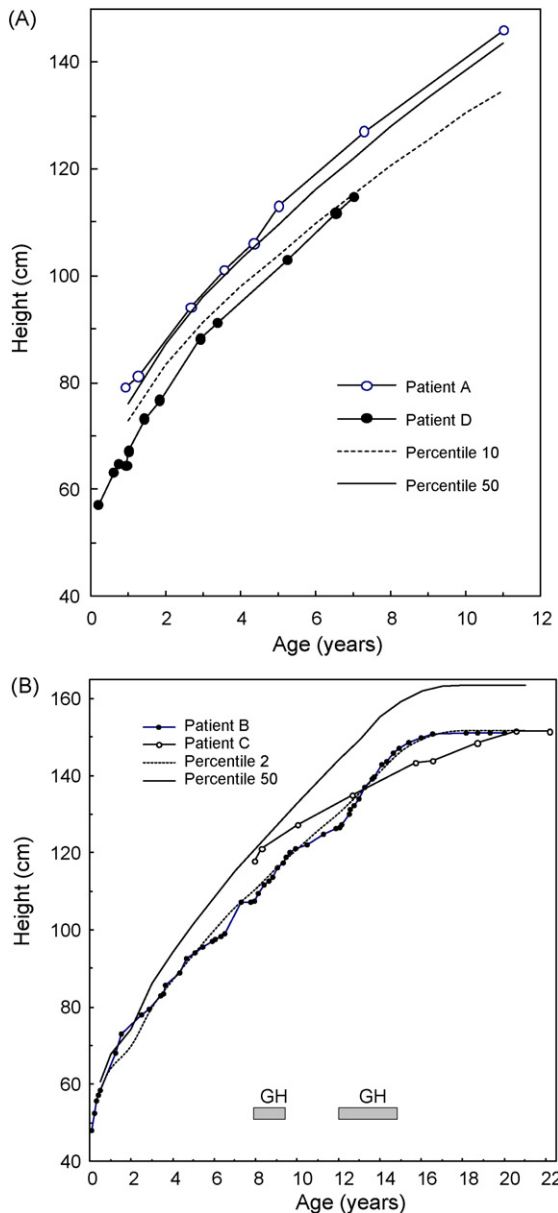| Patient	Age (year)	Sex	Height (cm)	Height SDS	Weight SDS	Pubertal status	Menses (year)	NaCl (g/day)
A	11.0	M	146.0	0.4	0.9	P1, T2	–	8
B	20.0	F	151.1	−2.1	0.4	P5, B5	14.2	15–20
C	22.1	F	151.5	−1.8	−0.1	P3, B2	None	<10
D	7.0	M	114.5	−1.1	−1.5	P1, T1	–	16

Fig. 4. Height-for-age growth-charts for (A) male patients A and D; and (B) female patients B and C. The gray bars mark the duration of GH treatment for only patient B

tion affects the pattern of current voltage relationship and the ion selectivity of ENaC, we carried out measurements in the presence of Na^+ or Li^+. The results of the I–V dependence studies showed that the Gly327Cys mutation significantly lowers both Li^+ and Na^+ ion currents (Fig. 6).

4. Discussion

In this study for the first time we examined the long-term (up to young adulthood) renin–aldosterone response, urinary Na/K ratio and growth in multi-system PHA patients as compared to renal PHA cases. Our results revealed major differences between renal and multi-system PHA cases, and also differences among multi-system cases depending on the type of ENaC mutation (Table 2).

In multi-system PHA patients, the long-term activity of the renin–aldosterone axis depended on the severity of salt-wasting. While in patient A renin–aldosterone axis activity decreased with
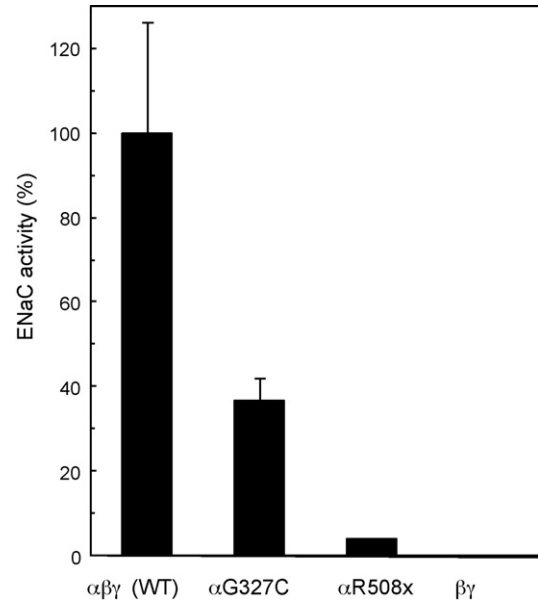

Fig. 5. Activity of different combinations of ENaC subunits expressed in Xenopus oocytes. Oocytes were injected with cRNAs that encode for the following ENaC sub-units: (1) αβγ: normal (WT) α, β and γ; (2) αGly327Cys: mutated αGly327Cys and normal β and γ; (3) mutated αArg508stop with normal β and γ (values from Bonny et al. [9]); and (4) normal β and γ only, without alpha. The amiloride-sensitive sodium current was measured by the two-electrode voltage-clamp method 2 days after injection. Results shown are the mean ± S.E.M. of nine oocytes per condition. The activity of ENaC with αGly327Cys was significantly different from the WT ($P < 0.02$).
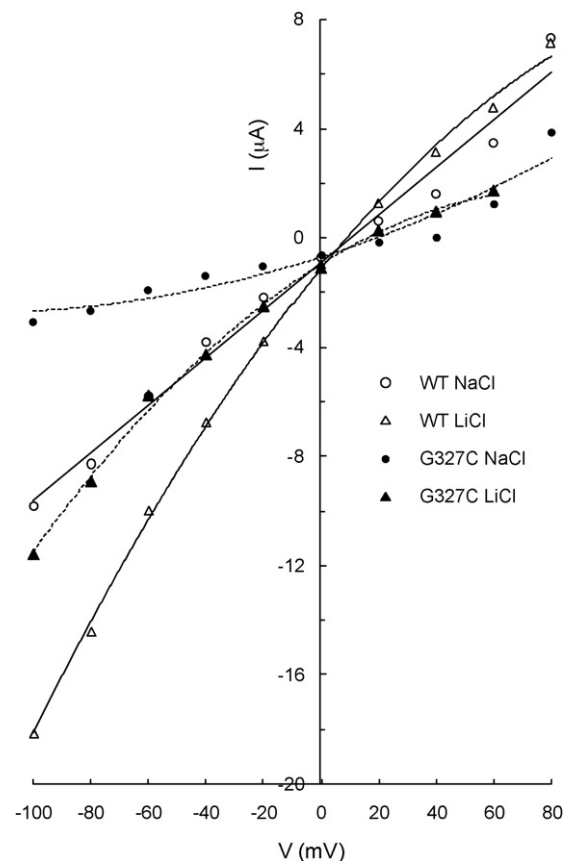

Fig. 6. Current–voltage relationship of currents recorded from Xenopus oocytes expressing WT ENaC and ENaC with αGly327Cys mutation (as in patient A). Current was recorded in the presence of 96 mM Na^+ (circles) or 96 mM Li^+ (triangles).

Table 2
Age related changes in serum aldosterone, plasma renin activity (PRA) and urinary Na/K ratio in multi-system and renal PHA patients between 2 years to young adulthood

	Patient	ENaC genotype	Aldosterone	PRA	Urinary Na/K	Amelioration with age[a]
Normal			0.06–0.4 nmol/L	0.5–3 ng/mL h	~2	–
Multi-system PHA	A	αGly327Cys αHis450fs	Decrease	Decrease	Decrease	Yes (after infancy)
	B	αArg508 Stop	Increase (4–300-fold)	Increase (3–25-fold)	Decrease	Yes (after 8–9 years)
	C	NA	Increase (40–400-fold)	Increase (3–37-fold)	Decrease	Yes (after 10 years)
	D	β1669 + 1G → A	Increase (6-fold)	Increase (3-fold)	Decrease	Yes (after 6 years)
Renal PHA	E	Normal	Persistently high (2–20-fold). No change with age	Decrease to normal	Normal no change	Yes (asymptomatic after 3 years)
	F	Normal	Persistently high (2–7-fold). No change with age	Decrease to normal	Normal no change	Yes (asymptomatic after 3 years)
	Adults (Ref. [13])	Normal	High (2–14-fold)	NA	Normal FENa	NA

FENa: fractional excretion of sodium; NA: not available.
Fold increase values are relative to upper level of normal range.

[a] Amelioration of salt-wasting episodes and respiratory infections.

age, reaching near normal values, in the more severe cases a dramatic increase was observed. This difference suggests that the hyper-activation of the renin–aldosterone system is a response to severe chronic salt-loss from multiple target organs in the severe cases who require high amounts of salt in their diet (Table 1). The chronic salt-loss is also probably associated with hypovolemia that may also trigger aldosterone secretion. The renal PHA cases do not require Na supplementation beyond the age of 2–4 years and their aldosterone–renin response remains stable. This provides further evidence that the hyper-activation phenomenon in multi-system PHA cases reflects the severity of the salt-loss. Our results showed strong correlation between serum aldosterone and PRA in the severe multi-system PHA patients. These findings indicate that the very high aldosterone levels result from activation of the renin-angiotensin system, and not from an autonomous activation of zona glomerulosa as in tertiary hyper-aldosteronism. Previous studies have shown that there is a strong correlation between PRA and angiotensin II in children and young adults [22,23].

The differences between the mild case of patient A and severe cases B, C and D are explained by our results of the functional expression of mutated ENaC subunits. ENaC with αGly327Cys mutation, as observed in patient A, showed nearly 40% activity of the wild type ENaC (Fig. 5). The mutated residue αGly327 is located in a conserved segment of ENaC-DEG-ASIC family of proteins. In the recently determined structure of chicken acid-sensing ion channel, the position of αGly327 corresponds to Gly218 that is located at the beginning of a β-strand in the palm domain of the protomer [24]. The preceding residue, Asn217, is close to the neighboring subunit. It is possible that the R-group of Cys that is larger than the Glycine327 weakens the binding of the subunits leading to reduced ENaC activity. It should be noted that αGly327Cys mutation is a heterozygous mutation in patient A, whose second mutation is His450fr [11]. Thus, only one allele of patient A encodes a partly functional αENaC. In contrast to the missense mutation, R508stop mutation, as observed in our patient B, results in a drastic reduction of ENaC activity to less than 5% activity of the wild type ENaC [9].

All multi-system PHA patients examined by us had increased urinary Na/K ratios reflecting impaired response to aldosterone. They also showed a surprising age-dependent decrease towards normal values in this ratio despite non-functional or impaired ENaC. The age-dependent improvement in urinary Na/K ratio was accompanied by amelioration of the clinical course of the patients with a significant decrease in the frequency and severity of salt-wasting episodes and respiratory tract infections that require hospitalization. Patient A showed the lowest urinary Na/K ratio throughout follow-up, consistent with the partial activity of ENaC with the missense αGly327Cys mutation.

The age-dependent normalization in the urinary Na/K ratio can not be attributed to a change in dietary salt consumption, since our patients consume large amounts of NaCl that would increase urinary Na/K ratio. The amount of salt consumed by the patients remained generally stable over the years. The improvement was also seen in patient C who was not compliant with therapy. In the renal PHA cases urinary Na/K ratio reached normal range after neonatal period and remained stable over the years.

The physiological mechanism responsible for the age-dependent decrease in urinary Na/K ratios in multi-system PHA patients is not known. In the severe cases there is a negative correlation between aldosterone levels and urinary Na/K ratio. However, this does not necessarily reflect a causal relationship. Aldosterone has inductive effects on a number of different proteins involved in ion transport, in addition to ENaC subunits. In A6 cells aldosterone has been shown to increase the expression of Na^+-K^+-ATPase [25]. However, in a study on PHA patients red blood cell activity Na^+-K^+-ATPase was found to be low [16], but this does not necessarily reflect the levels of this important pump in kidney and other relevant organs. Aldosterone has been reported to regulate Na–K–Cl co-transporter isoform NKCC1 in vascular smooth muscle [26] but it is not known whether it also regulates the NKCC2 isoform found in the kidney [27]. Whether these transporters or others are involved in the long-term adaptive response to compensate for the salt-loss observed in multi-system PHA remains to be determined.

Studies in normal subjects suggested that the urinary Na/K ratio reflects short-term (days) mineralocorticoid activity [28]. Our results show that this ratio is a reliable long-term indicator of aldosterone activity in PHA patients.

All severe cases of PHA with nonfunctional ENaC, were characterized by poor growth and pubertal delay. The most significant impairment was observed in patient C with poor compliance who showed significant growth and pubertal delay. In contrast, beyond neonatal period, patient A with a missense mutation showed a completely normal growth pattern. In patient B who showed deceleration in her height despite good compliance with therapy we diagnosed GH deficiency at 7.8 years. With GH therapy her final height reached close to her target height. This case represents the first long-term documentation of GH deficiency in a multi-system PHA patient. We thus suggest testing multi-system PHA patients who exhibit decreased growth rate for GH deficiency. Two of the young adult patients were relatively overweight despite growth impairment, implying that chronic salt-loss impairs predominantly the linear growth rather than weight.

In conclusion, the present study demonstrates distinct genotype–phenotype relationships in multi-system PHA patients.

The degree of ENaC function impairment affects differently the renin–aldosterone system and urinary Na/K ratios. The differences observed are age-dependent and PHA form specific, as the renal PHA patients present a different picture. The hyper-activation of the renin–aldosterone system with age was observed only in multi-system PHA patients with loss-of-function mutations and severe clinical course and not in a patient with an alpha-Gly327Cys missense mutation that reduces but does not eliminate ENaC function. Markedly reduced channel activity results in impaired linear growth and delayed puberty.

Acknowledgments

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