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ORIGINAL ARTICLE

# Novel mutations in epithelial sodium channel (ENaC) subunit genes and phenotypic expression of multisystem pseudohypoaldosteronism

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## Summary

**Objectives** Multisystem pseudohypoaldosteronism (PHA) is a rare autosomal recessive aldosterone unresponsiveness syndrome that results from mutations in the genes encoding epithelial sodium channel (ENaC) subunits α, β and γ. In this study we examined three PHA patients to identify mutations responsible for PHA with different clinical presentations.

**Patients** All three patients presented uniformly with symptoms of severe salt-loss during the first week of life and were hospitalized for up to a year. Beyond infancy, one of the patients showed mild renal salt loss and had no lower respiratory tract infections until 8 years of age, while the other patients continue with a severe course.

**Results** We sequenced the complete coding regions and intron–exon junctions of the genes encoding α, β and γ subunits of ENaC for all patients. The results revealed that the mild case represents a novel compound heterozygote including a missense (Gly327Cys) mutation in the αENaC gene. Sequences of relatives over three generations confirmed that the missense mutation co-segregates with PHA. This mutation was not found in 60 control subjects. The other patients with severe PHA had two homozygous mutations, a novel deletion mutation in exon 8 of the αENaC gene and a splice site mutation in intron 12 of the βENaC gene. Most of the PHA-causing mutations appear in the αENaC gene located on chromosome 12 rather than in the β and γENaC genes located tandemly on chromosome 16. However, the frequency of sequence variants in patients and control subjects showed no difference between genes.

**Conclusions** Severe PHA cases are associated with mutations leading to absence of normal-length α, β or γENaC, while a mild case has been found to be associated with a missense mutation in αENaC. The predominance of PHA-causing mutations in the αENaC gene may be related to the function of this subunit.

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## Introduction

Pseudohypoaldosteronism type I (PHA) is a syndrome of unresponsiveness to aldosterone that is expressed in two distinct forms: renal PHA and multisystem PHA.[1] While the renal form generally results from autosomal dominant mutations in the mineralocorticoid receptor,[2,3] multisystem PHA results from autosomal recessive mutations in the genes encoding epithelial sodium channel (ENaC) subunits.[4–11]

ENaC is composed of three related subunits (α, β and γ) encoded by three genes located on chromosomes 12 and 16.[4,6,12–14] ENaC subunits are expressed in epithelial cells lining the renal tubule, respiratory airways, the distal colon and the ducts of several exocrine glands, such as salivary and sweat glands. ENaC plays an important role in electrolyte homeostasis and the control of blood volume and blood pressure.[14,15]

In the absence of a fully functional ENaC, multisystem PHA patients lose salt from all aldosterone-dependent epithelial cells. The clinical and laboratory characteristics of multisystem PHA include severe and life-threatening episodes of salt wasting with extreme hyperkalaemia, hyponatraemia, dehydration, failure to thrive and markedly elevated levels of aldosterone and plasma renin activity (PRA).[1,7–11,16] Disease symptoms manifest during the first week of life and require prolonged hospitalizations. Salt-wasting episodes recur frequently and the patients need life-long high-salt therapy (iv or dietary salt supplementation up to 45 g/day). The mortality rate is high, especially during the neonatal period.

Besides the usual symptoms of salt wasting, we have reported that the multisystem PHA patients also frequently exhibit respiratory tract disease, especially up to the age of 5–6 years.[17] The cause of these pulmonary manifestations was shown to be excess liquid in the airways due to inadequate absorption of Na and water because of defective ENaC function.[7] These phenotypic features exist uniformly in all affected patients. The question of possible phenotype–genotype relationships in PHA has not been addressed in previous studies. This is probably because of the rarity of the syndrome and lack of long-term follow-up evaluation of PHA patients.

In this study, we have examined three independent patients with multisystem PHA who share the basic common characteristics of the disorder. Beyond the neonatal period, long-term (up to 8·3 years) prospective evaluation of two of the patients showed significant differences in the severity and the course of disease. Our findings revealed different types of mutations that help to explain the different phenotypic expression of multisystem PHA. We examine and classify all previously reported cases in the light of the phenotypic and genotypic differences we observed in our new patients.

## Subjects and methods

### *Subjects*

The backgrounds of the PHA patients are presented together with their clinical description. The control population for examination of the frequency of the novel missense mutation included 60 normal subjects from Szczecin and Katowice regions in North and South Poland, respectively. Patients and control individuals whose entire coding regions of α, β and γ ENaC genes were sequenced represent the following ethnic groups: Israeli-Arab, Belgian, Canadian, Jewish (Sephardic), Polish and Turkish.

### *Genomic DNA isolation, PCR and sequencing*

Genomic DNA of the patients and their relatives was extracted from white blood cells as described previously.[13] The DNA samples of the control subjects were isolated from the umbilical blood of a contiguous series of neonates.

Segments of α, β and γ ENaC genes were amplified using previously described primer sets[13] and additional primer sets shown in Table 1. Polymerase chain reaction (PCR) was carried out using a Taq polymerase kit (Takara, Japan). PCR products were run on 2% agarose gel, extracted and purified using QIAquick Gel Extraction kit (Qiagen, Germany). Sequencing reactions were carried out using dye terminators (ABI). Dye terminators were removed using DyeEx Spin kit (Qiagen) and the DNA sequences were analysed in our laboratory using an ABI automated DNA sequencer.

## Results

### *Clinical course of PHA patients*

All three PHA cases described below followed a severe course of multisystem PHA during the neonatal period with increased concentrations of sodium and chloride in sweat and saliva (Table 2). Patient 44 followed a clinical course that differed significantly beyond this period. He was born at full term to healthy nonconsanguineous Polish parents (birthweight 4·1 kg). He presented at the age of 3 days with severe electrolyte disturbances, poor feeding, vomiting and weight loss. His younger brother aged 2 years 8 months is healthy. Laboratory examinations showed marked hyponatraemia, hyperkalaemia and metabolic acidosis (pH 7·3; $HCO_3$, 13·7 mmol/l). Episodes of hypovolaemic shock and severe electrolyte imbalance persisted for 2 weeks despite therapy with iv fluids, bicarbonate and NaCl. Laboratory evaluation revealed persistent hyperaldosteronism and hyper-reninaemia with urinary salt wasting and increased Na/K ratios (Table 2). Other renal and adrenal functions were normal. He gradually improved with iv NaCl therapy (11 g/day) and cation exchange resin (1 g/kg/day). He was discharged at the age of 4·5 months.

After discharge he remained free of salt-wasting episodes and lower respiratory tract infections and did not require hospitalization throughout the entire follow-up period. Laboratory data were consistent with milder salt wasting. During follow-up visits every 1–6 months (Table 3), electrolyte levels were within the normal range except for an isolated episode of mild hyponatraemia and hyperkalaemia at 5 months of age (Na, 133 mmol/l; K, 6·4 mmol/l). His urinary sodium/potassium ratio remained significantly lower than the ratio observed in the other two patients (Table 2). Sweat and saliva electrolytes ($N = 8$) remained persistently elevated (Table 2). Cation exchange resin was discontinued at the age of 15 months. Currently, at 8·3 years of age he is still on a high-salt diet (8 g NaCl/day) and is growing normally.

Patient 66 was born after a normal pregnancy (birthweight 3·2 kg). His parents are Israeli Arabs whose grandparents were first cousins. The parents and two brothers aged 8 and 4 are healthy. A younger sister died at the age of 3 months after a short illness. The patient was hospitalized at 6 days of age because of a shock-like episode and severe dehydration. Laboratory findings showed marked hyperkalaemia, hyponatraemia, acidosis (pH 7·2) and increased urinary

**Table 1.** New primer sets used to amplify and sequence exons and introns of the human α, β and γ ENaC genes

| Primer | Exon | Forward | Reverse | Temperature (°C) |
|---|---|---|---|---|
| A5F–A5RO | 4 | cctctgactctagtctctgtgtc | agagcaaggagccaggcagga | 65 |
| A5aR | 5 | | taggaggtgagctcaaggta | 60 |
| A6aF | 6 | tcttcctgaacttgcttctc | | 55 |
| B10FO–B10RO | 10 | cgcagagagagacctgcatt | agacttgtccccagccaaag | 64 |
| G2FO–G2·1RO | 2 | aagggcggatggactggtgt | gcgtttccctgctcatgcct | 65 |
| G11F–G12R | 13 | ttgatggtgtggcttggcctg | gatctgtcttctcaaccctgc | 60 |

**Table 2.** Biochemical characteristics of multisystem PHA patients at first admission and during hospitalization and follow-up

| Patient | 44 In-patient | 44 Out-patient | 66 In-patient | 66 Out-patient | TR2 In-patient | TR2 Out-patient | Normal |
|---|---|---|---|---|---|---|---|
| Age | 3 days–4·5 months | 4·5 months–8·3 years | 6 days–4·5 months | 4·5 months–3·5 years | 8 days–25 days | 25 days–5 months | |
| Serum Na (mmol/l) | 127 (116–141) | 140 (133–146) | 135 (126–143) | 138 (125–147) | 128 (113–144) | 136 (135–139) | 136–146 |
| Serum K (mmol/l) | 7·6 (4·6–9·5) | 4·8 (4·4–6·4) | 5·9 (3·2–10·6) | 4·6 (3·1–6·8) | 6·8 (3·2–9·5) | 4·7 (4·1–5·2) | 3·5–5 |
| Serum aldosterone* (pmol/l) | 16 491 (14 563–18 419) | 10 208 (6796–19 170) | 1110 to > 6935 | 5172 (55–10 290) | 12 259 | – | 55–388 |
| PRA (ng/l s)* | 12·5 (7–18) | 10·4 (3·8–18·5) | > 50 | 23 (3–43) | > 500† | – | < 3 |
| Sweat Cl (mmol/l) | 91 (90–92) | 108 (80–120) | – | 220 | 140 | – | < 50 |
| Salivary Na (mmol/l) | – | 69 (50–95) | – | 131 (95–157) | – | 88 | < 50 |
| Salivary Cl (mmol/l) | – | 71 (42–110) | – | 138 (129–146) | – | 56 | < 50 |
| Urine Na (mmol/l) | 93 (60–143) | 199 (198, 200) | 107 (38–232) | 189 (101–266) | 101 (76–126) | 82 | < 40 |
| Urine Na/K ratio | 11 (6·7–16) | 4·3 (3·8–4·8) | 290 (38–1780) | 40 (7·4–84) | 26 (14–33) | 41 | *ca.* 2 |

The numbers in parentheses are the range of values. *Normal aldosterone and PRA in infants younger than 3 months: aldosterone < 3051 pmol/l; PRA < 10 ng/l s. †Renin level in patient TR2 is expressed in ng/l (normal: 2·4–21·9).

**Table 3.** Principal differences between mild (patient 44) and severe phenotypes of multisystem PHA beyond 5 months of age

| | Phenotype | |
|---|---|---|
| | Mild | Severe |
| Salt wasting | Mild | Severe |
| Hospitalization | None | Frequent |
| Respiratory illness | No | Yes |
| Growth failure | No | Yes |
| Therapy | NaCl, Kayexalate | NaCl, sodium bicarbonate Kayexalate, gastrostomy |
| Mortality | No | High risk |
| Mutations | Compound heterozygote (missense and deletion) | See Table 4 |

sodium (47 mmol/l) and Na/K ratio (Table 2). He was treated with iv saline, and Kayexalate. Aldosterone level and PRA were markedly elevated. Renal, adrenal and thyroid function tests (renal ultrasound, basal and ACTH-stimulated 17-hydroxyprogesterone and cortisol, free T4 and TSH levels) were normal. Urinary Na and Na/K ratios remained high (Table 2). Frequent episodes of salt wasting and marked hyperkalaemia (up to 10 mmol/l) necessitated prolonged hospitalization. He was discharged at the age of 4·5 months. Recurrent episodes of vomiting and dehydration persisted (hospitalizations every 1–3 months). To prevent life-threatening salt-wasting episodes, gastrostomy was performed at 14 months of age to provide high amounts of NaCl (20 g/day) and improve his caloric intake. Additional clinical characteristics included persistent clear nasal discharge, frequent lower respiratory infections associated with wheezing, and failure to thrive. Following gastrostomy, the number of hospitalizations decreased significantly (1–2 per year). However, he still gets lower respiratory infections. Currently, at the age of 4 years he is still on a high-salt diet (15 g NaCl/day) by gastrostomy and Kayexalate.

Patient TR2 was born at term and delivered by caesarean section. Her birthweight was 3·65 kg. Her parents are first cousins. A brother and sister died at the age of 1 week after a fulminant, undiagnosed illness characterized by vomiting. A 17-year-old sister has been followed up for congenital hepatic fibrosis. The patient was transferred to a neonatal intensive care unit at 8 days of age with a 1-day history of vomiting. Physical examination revealed a mildly dehydrated infant, weighing 3·4 kg. Laboratory examination showed marked hyponatraemia and hyperkalaemia with urinary salt wasting. PRA and aldosterone levels were extremely high (Table 2). Renal and adrenal functions tests were otherwise normal. She was treated with iv NaCl and Kayexalate. Because of extremely high potassium levels, peritoneal dialysis was also performed. Currently, at 5 months of age, she is on a high-salt diet (3 g NaCl/day) and Kayexalate (4 g × 3/day) and is developing normally. Her electrolytes are now normal (Table 2).

### *PHA-associated mutations*

We sequenced the complete coding regions and intron–exon junctions of the α, β and γ ENaC subunit genes of the PHA patients. In patient 44,

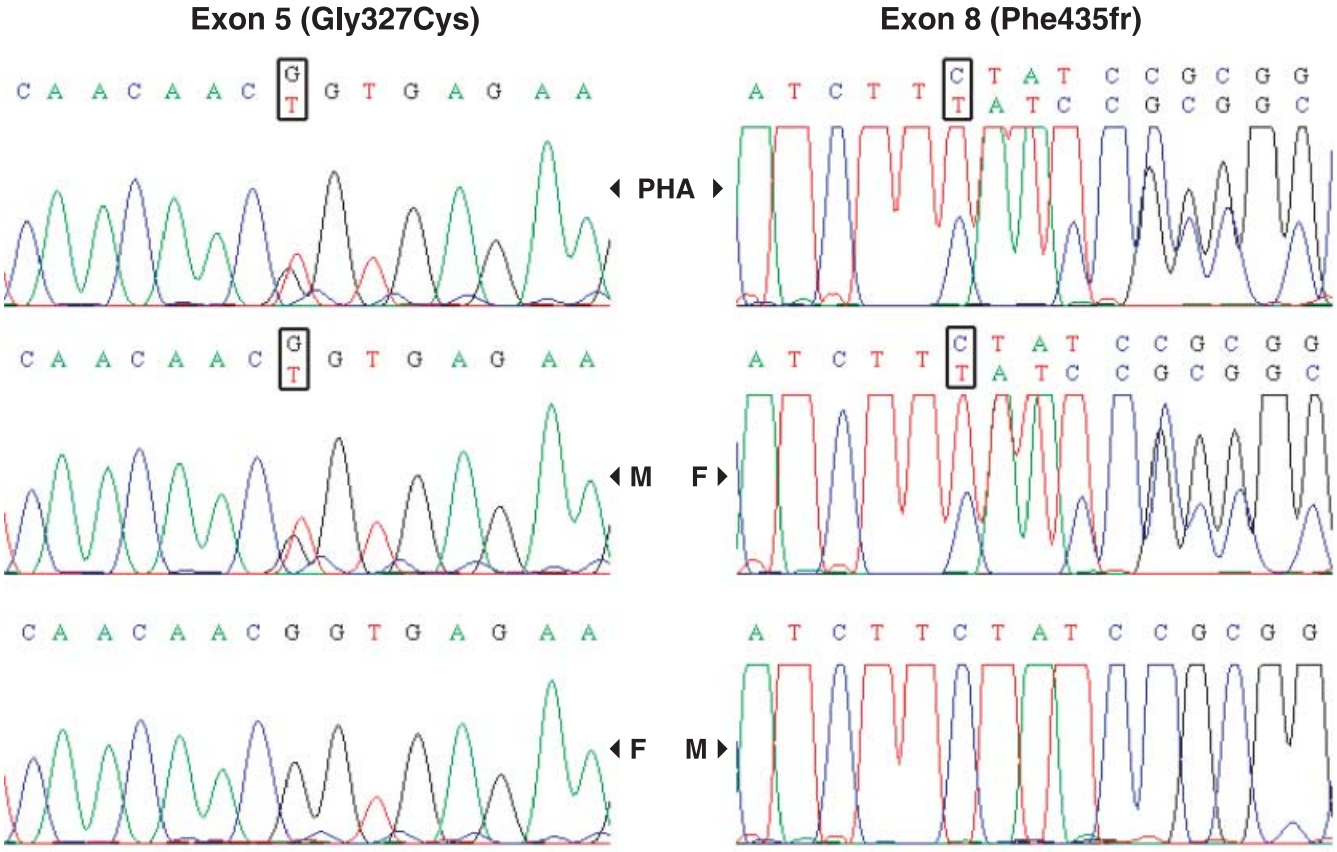


**Fig. 1** Compound heterozygote mutations in the gene encoding the αENaC subunit in family 44. Left panel: Exon 5 region missense mutation: 1078 G→T, Gly327Cys. The sequences for both the mother (M) and PHA son show heterozygosity with G and T at the same location (boxed), while the father's sequence is normal. Right panel: Exon 8 region deletion mutation: 1404delC, Phe435fr. The sequences for both the father (F) and PHA son show heterozygosity with a deletion at the same location (boxed), while the mother's sequence is normal.

we observed two heterozygous mutations in exons 5 and 8 of αENaC (Fig. 1, Table 4). The first mutation was found in the last nucleotide of exon 5 (1078 G→T), which converted GGT codon coding for glycine-327 into a TGT codon coding for cysteine. Thus, this allele encodes a protein with a missense mutation (Gly327Cys). The mutation at exon 8 (1404delC) was a deletion that would result in a frameshift mutation (Phe435fr) (Fig. 1). Sequencing the genomic DNA of both parents and maternal and paternal grandparents revealed that the mutation in exon 5 was inherited from the maternal grandfather, and the mutation in exon 8 was inherited from the paternal grandfather (Figs 1 and 2). Genomic DNA of a sibling who shows no signs of PHA showed a normal sequence without these heterozygosities, indicating that he inherited the normal alleles from both parents. Sequencing of αENaC exon 5 from 60 normal Polish control subjects did not show any mutation in this region. The mutated Gly327 appears in the extracellular domain of αENaC in a region that is strictly conserved in all known sequences of ENaC from eight species, including Xenopus (Fig. 3).

For patient TR2 sequencing revealed a novel deletion mutation in exon 8 of the αENaC gene at Ser452 codon, which results in a frameshift error. Genomic DNAs of both parents showed heterozygosity at this position, confirming that the patient inherited the mutated allele from both parents (Fig. 4).

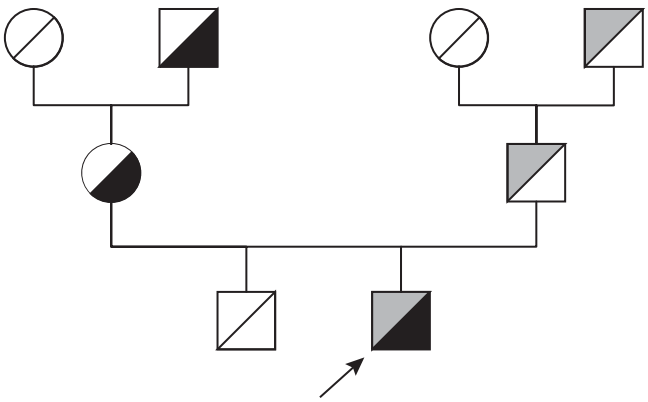

**Fig. 2** Pedigree of family 44 showing inheritance pattern of mutations. The mother and maternal grandfather carry a heterozygous missense mutation (black) in αENaC exon 5 (1078G→T, Gly327Cys). The father and paternal grandfather carry a heterozygous deletion mutation (shaded) in αENaC exon 8 (1404delC, Phe435fr). Normal alleles are shown in white. The arrow marks the propositus.

For patient 66, sequencing analysis revealed a homozygous mutation of G to A in the first nucleotide of intron 12 of the βENaC gene. This mutation changed the conserved GT pair at the 5′ end of the intron resulting in abnormal splicing of the βENaC mRNA, and

**Table 4.** Mutations identified in coding regions of ENaC subunit genes in patients with autosomal recessive multisystem PHA

| Subunit | Location | Mutation | Codon change | Phenotype | Ethnicity | Reference |
|---|---|---|---|---|---|---|
| Missense mutation | | | | | | |
| Alpha | Exon 5 | 1078 G→T | Gly327Cys* | Mild* | Polish | This study |
| Alpha | Exon 13 | 1784C→T | Ser562Leu* | Mild?* | Swedish | Schaedel *et al.*[8] |
| Beta | Exon 2 | 236 G→A | Gly37Ser | ? | Arab | Chang *et al.*[4] |
| Non-missense mutation | | | | | | |
| Alpha | Exon 2 | 256 C→T | Arg53stop | ? | Northern Europe | Kerem *et al.*[7] |
| Alpha | Exon 2 | 302delTC | Ile68fr | Severe | Saudi | Chang *et al.*[4] |
| Alpha | Exon 3 | 604delAC | Thr169fr | Severe | Hispanic | Kerem *et al.*[7] |
| Alpha | Exon 4 | 828delA | Ser243fr | Severe | Swedish | Schaedel *et al.*[8] |
| Alpha | Exon 8 | 1404delC | Phe435fr | Severe | Hispanic | Kerem *et al.*[7] |
| Alpha | Exon 8 | 1439insT | Tyr447fr | Severe | Pakistani | Saxena *et al.*[11] |
| Alpha | Exon 8 | 1449delC | His450fr* | Mild* | Polish | This study |
| Alpha | Exon 8 | 1449delC | His450fr | Mild/Severe† | Swedish | Schaedel *et al.*[8] |
| Alpha | Exon 8 | 1455delC | Ser452fr | NLT | Turkish | This study |
| Alpha | Exon 10 | | Arg492stop | Severe | Dutch? | Bonny *et al.*[10] |
| Alpha | Exon 11 | 1621C→T | Arg508stop | Severe | Indian Muslim | Saxena *et al.*[11] |
| Alpha | Exon 11 | 1621C→T | Arg508stop | Severe | Jewish (Iranian) | Chang *et al.*[4] |
| | | | | | | Kerem *et al.*[7] |
| Beta | Exon 3 | 647insA | Leu174fr | ? | Jewish (Ashkenazi) | Kerem *et al.*[7] |
| Beta | Exon 5 | 915delC | Ser263fr | ? | Jewish (Ashkenazi) | Kerem *et al.*[7] |
| Beta | Intron 12 | 1669 + 1G→A | Abnormal splicing | Severe | Arab | This study |
| Beta | Intron 12 | 1669 + 1G→A | Abnormal splicing | Severe | Scottish | Saxena *et al.*[11] |
| Gamma | Intron 2 | 318 − 1G→A | Abnormal splicing | NLT | Indian | Strautnieks *et al.*[6] |
| Gamma | Intron 12 | 1570 − 1G→A | Abnormal splicing | Severe | Japanese | Adachi *et al.*[9] |
| Gamma | Exon 13 | 1627delG | Val543fr | Severe | Japanese | Adachi *et al.*[9] |

*Compound heterozygote including a missense mutation. †Mild phenotype in association with missense. Severe phenotype in association with another deletion mutation. NLT, no long-term clinical data.

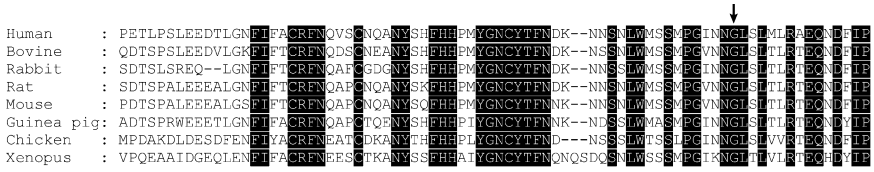


**Fig. 3** Comparison of the sequences of αENaC from eight species. The arrow marks the position of the Gly327Cys mutation. Note that glycine at this position is conserved in all species. The segment of the human sequence starts at Pro-264 and ends at Pro-341. Sequences from other species were obtained from the Swiss-Prot protein database.

consequently absence of normal βENaC subunit. Genomic DNAs of both parents showed heterozygosity at this position, confirming that the patient inherited the mutated allele from both parents (data not shown).

For all three patients noted above, sequencing of all three subunit genes did not reveal additional changes that could be associated with PHA. The sequencing of the entire coding regions of all three subunit genes in the three patients and in four additional subjects representing altogether five different ethnic groups revealed only a few phenotypically silent sequence variants different from previous sequences (Table 5).

**Table 5.** Frequencies of sequence variants observed in all three ENaC subunit genes whose coding regions were sequenced entirely for seven independent subjects

| | αENaC SCNN1A | βENaC SCNN1B | γENaC SCNN1G |
|---|---|---|---|
| Chromosome | 12 | 16 | 16 |
| Variants | 2 | 2 | 2 |
| Length (bp) | 2007 | 1920 | 1947 |
| Frequency/1000 bp | 0·997 | 1·042 | 1·027 |

Entrez Accession codes for reference sequences: SCNN1A: X76180; SCNN1B: AJ005383; SCNN1G: AF356502.

## Discussion

In this study we have identified four mutations responsible for multisystem PHA in three new patients. Together with the findings presented here, world-wide there are 22 independent mutations known in the coding regions of ENaC subunit genes (Table 4).

The majority (19 out of 22) of the multisystem PHA-associated mutations led to abnormal-length mRNA or protein as a result of deletion, insertion or splice site mutations (Table 4). Without exception, all these mutations are associated with the severe phenotype of

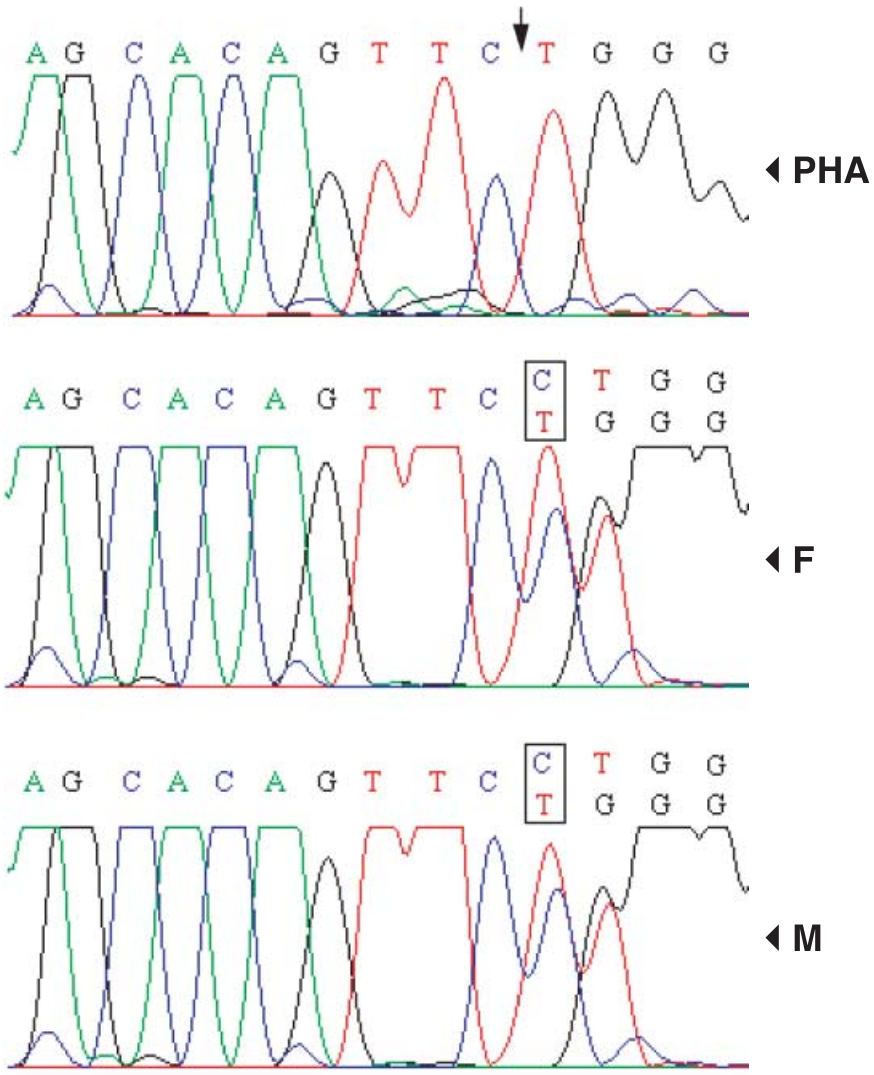


**Fig. 4** Sequence of the αENaC gene exon 8 segment with a deletion mutation in patient TR2. The top sequence belongs to patient TR2. The arrow marks the position of the deleted nucleotide C. The bottom two sequences are of the father and mother, respectively. Both sequences show heterozygosity with a normal allele (AGCACAGTTCCTGG) and an abnormal allele with a deletion (AGCACAGTTCTGGG). Sequencing was carried out using primer A9F.

PHA (Table 2) that matches the first description of multisystem PHA.[1]

Only three out of the 22 known mutations are missense mutations. Our patient with the missense mutation represents the first well-documented PHA patient with long-term follow-up (over 8 years) who had a mild form of PHA unlike other PHA patients (Table 3). Beyond infancy, while the clinical course of patient 44 was characterized by mild renal salt wasting, absence of lower respiratory tract symptoms and normal growth, patient 66 exhibited recurrent salt-wasting episodes and pulmonary infections throughout follow-up. Therapeutic measures necessary to prevent salt wasting were also milder in patient 44 relative to the severe cases. The patient did not require Kayexalate after the age of 15 months, and dietary NaCl supplementation was sufficient to normalize electrolytes allowing normal growth. The patient with the αENaC Ser562Leu mutation was also reported to show mild pulmonary manifestations, but the extent of renal salt wasting was not reported.[8] The report for the third patient with a Gly37Ser mutation does not have sufficient clinical data for classification.[4]

The observation of a milder phenotype for missense as opposed to a severe phenotype for nonmissense mutations in ENaC subunit genes is understandable. While the nonmissense mutations lead to absence of normal-length protein, missense mutations allow synthesis of a normal-length subunit that is more likely to support channel activity than an absent subunit. Schaedel *et al.*[8] suggested that lung symptoms in PHA are associated with defective αENaC subunits rather than βENaC or γENaC. However, frequent pulmonary diseases have been described recently in a 7-year-old Japanese child with compound heterozygous mutations in the γENaC subunit gene.[9] Our patient 44 with a mutation in the αENaC subunit had no recurrent pulmonary infections. Thus, present data do not support a specific association of only the αENaC subunit with the severe phenotype.

In the absence of *in vitro* expression studies on the mutated subunit we do not know the precise effect of the mutation on the ENaC structure and function. The Gly327Cys mutation is located in the extracellular domain of αENaC (see compared sequences[13]). Thus, our results indicate that the extracellular domain is important for the function of the subunit. Understanding the specific structural

and functional consequences of the mutation awaits determination of the structure of this domain.

The majority of the multisystem PHA mutations (14 out of 22) appear on the αENaC gene (Table 4). Within the αENaC gene the mutations appear more frequently in exon 8. It still remains to be seen whether this tendency will persist as more novel mutations are discovered. The frequencies of phenotypically silent sequence variants are low and do not show a difference among the different subunit genes located on different chromosomes (Table 4). The number of single nucleotide polymorphism (cSNP) in the NCBI SNP database (http://www.ncbi.nlm.nih.gov/SNP/) is 7, 12 and 10 for the α, β and γ subunits, respectively. In addition to the coding region mutations noted in Table 4, a PHA associated mutation has also been reported in the promoter region of the αENaC gene.[18]

The deletion in exon 8 (1404delC) was previously observed in a Swedish patient with multisystem PHA in combination with a different mutation.[8] We do not know whether our finding of the same exon 8 mutation in a Polish family reflects a common origin.

In patient 66 of Arabic origin we identified a homozygous mutation causing abnormal splicing, identical to a mutation we recently reported in a Scottish patient.[11] This mutation should lead to the absence of normal mRNA for the βENaC subunit. The mutation is located at a CpG hotspot. Therefore, it does not necessarily indicate a common genetic origin for the mutations.

The new multisystem pseudohypoaldosteronism cases and mutations elucidated in this report provide a better understanding of the molecular bases of this disease and their phenotypic expression. The mutations provide clues about functionally important regions of epithelial sodium channel subunits.

## Acknowledgements

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## References

1 Hanukoglu, A. (1991) Type I pseudohypoaldosteronism includes two clinically and genetically distinct entities with either renal or multiple target organ defects. *Journal of Clinical Endocrinology and Metabolism*, **73**, 936–944.

2 Zennaro, M.C. & Lombes, M. (2004) Mineralocorticoid resistance. *Trends in Endocrinology and Metabolism*, **15**, 264–270.

3 Tajima, T., Kitagawa, H., Yokoya, S., Tachibana, K., Adachi, M., Nakae, J., Suwa, S., Katoh, S. & Fujieda, K. (2000) A novel missense mutation of mineralocorticoid receptor gene in one Japanese family with a renal form of pseudohypoaldosteronism type 1. *Journal of Clinical Endocrinology and Metabolism*, **85**, 4690–4694.

4 Chang, S.S., Grunder, S., Hanukoglu, A., Rosler, A., Mathew, P.M., Hanukoglu, I., Schild, L., Lu, Y., Shimkets, R.A., Nelson-Williams, C., Rossier, B.C. & Lifton, R.P. (1996) Mutations in subunits of the epithelial sodium channel cause salt wasting with hyperkalaemic acidosis, pseudohypoaldosteronism type 1. *Nature Genetics*, **12**, 248–253.

5 Strautnieks, S.S., Thompson, R.J., Gardiner, R.M. & Chung, E. (1996) A novel splice-site mutation in the gamma subunit of the epithelial sodium channel gene in three pseudohypoaldosteronism type 1 families. *Nature Genetics*, **13**, 248–250.

6 Strautnieks, S.S., Thompson, R.J., Hanukoglu, A., Dillon, M.J., Hanukoglu, I., Kuhnle, U., Seckl, J., Gardiner, R.M. & Chung, E. (1996) Localisation of pseudohypoaldosteronism genes to chromosome 16p12.2–13.11 and 12p13.1-pter by homozygosity mapping. *Human Molecular Genetics*, **5**, 293–299.

7 Kerem, E., Bistritzer, T., Hanukoglu, A., Hofmann, T., Zhou, Z., Bennett, W., MacLaughlin, E., Barker, P., Nash, M., Quittell, L., Boucher, R. & Knowles, M.R. (1999) Pulmonary epithelial sodium-channel dysfunction and excess airway liquid in pseudohypoaldosteronism. *New England Journal of Medicine*, **341**, 156–162.

8 Schaedel, C., Marthinsen, L., Kristoffersson, A.C., Kornfalt, R., Nilsson, K.O., Orlenius, B. & Holmberg, L. (1999) Lung symptoms in pseudohypoaldosteronism type 1 are associated with deficiency of the alpha-subunit of the epithelial sodium channel. *Journal of Pediatrics*, **135**, 739–745.

9 Adachi, M., Tachibana, K., Asakura, Y., Abe, S., Nakae, J., Tajima, T. & Fujieda, K. (2001) Compound heterozygous mutations in the gamma subunit gene of ENaC (1627delG and 1570–1G→A) in one sporadic Japanese patient with a systemic form of pseudohypoaldosteronism type 1. *Journal of Clinical Endocrinology and Metabolism*, **86**, 9–12.

10 Bonny, O., Knoers, N., Monnens, L. & Rossier, B.C. (2002) A novel mutation of the epithelial Na+ channel causes type 1 pseudohypoaldosteronism. *Pediatric Nephrology*, **17**, 804–808.

11 Saxena, A., Hanukoglu, I., Saxena, D., Thompson, R.J., Gardiner, R.M. & Hanukoglu, A. (2002) Novel mutations responsible for autosomal recessive multisystem pseudohypoaldosteronism and sequence variants in epithelial sodium channel alpha-, beta-, and gamma-subunit genes. *Journal of Clinical Endocrinology and Metabolism*, **87**, 3344–3350.

12 Canessa, C.M., Schild, L., Buell, G., Thorens, B., Gautschi, I., Horisberger, J.D. & Rossier, B.C. (1994) Amiloride-sensitive epithelial $Na^+$ channel is made of three homologous subunits. *Nature*, **367**, 463–467.

13 Saxena, A., Hanukoglu, I., Strautnieks, S.S., Thompson, R.J., Gardiner, R.M. & Hanukoglu, A. (1998) Gene structure of the human amiloride-sensitive epithelial sodium channel beta subunit. *Biochemical and Biophysical Research Communications*, **252**, 208–213.

14 Rossier, B.C., Pradervand, S., Schild, L. & Hummler, E. (2002) Epithelial sodium channel and the control of sodium balance: interaction between genetic and environmental factors. *Annual Review of Physiology*, **64**, 877–897.

15 Garty, H. & Palmer, L.G. (1997) Epithelial sodium channels: function, structure, and regulation. *Physiological Reviews*, **77**, 359–396.

16 Oberfield, S.E., Levine, L.S., Carey, R.M., Bejar, R. & New, M.I. (1979) Pseudohypoaldosteronism: multiple target organ unresponsiveness to mineralocorticoid hormones. *Journal of Clinical Endocrinology and Metabolism*, **48**, 228–234.

17 Hanukoglu, A., Bistritzer, T., Rakover, Y. & Mandelberg, A. (1994) Pseudohypoaldosteronism with increased sweat and saliva electrolyte values and frequent lower respiratory tract infections mimicking cystic fibrosis. *Journal of Pediatrics*, **125**, 752–755.

18 Thomas, C.P., Zhou, J., Liu, K.Z., Mick, V.E., MacLaughlin, E. & Knowles, M. (2002) Systemic pseudohypoaldosteronism from deletion of the promoter region of the human beta epithelial Na(+) channel subunit. *American Journal of Respiratory Cell and Molecular Biology*, **27**, 314–319.