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Novel Mutations Responsible for Autosomal Recessive Multisystem Pseudohypoaldosteronism and Sequence Variants in Epithelial Sodium Channel α-, β-, and γ-Subunit Genes

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Multisystem pseudohypoaldosteronism (PHA), is a syndrome of unresponsiveness to aldosterone with autosomal recessive inheritance. Previously we showed that mutations in the epithelial sodium channel (ENaC) α-, β-, and γ-subunits are responsible for PHA. In this study we examined four independent probands with multisystem PHA, three of whom were born to consanguineous parents. In our search for mutations we also determined the complete coding sequences of each of the three genes encoding α-, β-, and γ-subunits in individuals representing different ethnic groups. Our analyses revealed the following homozygous mutations in three probands: 1) insertion of a T in exon 8 of the α ENaC gene that causes a frameshift error at Tyr^{447} and leads to a premature stop codon at K459 in a Pakistani patient; 2) R508stop mutation in exon 11 of the α ENaC gene in an Indian patient; and 3) a splice site mutation in intron 12 of the β ENaC gene (1669 + 1 g→a) in a Scottish patient. The parents were heterozygous for the latter two mutations. The second mutation was previously observed in an Iranian Jewish patient. Our sequencing of the α-, β-, and γ-coding sequences revealed some sequence variants, some of which may represent single nucleotide polymorphisms. The γ-subunit protein sequence was completely conserved in the six subjects examined. The homozygous mutations identified in the α and β ENaC genes should result in reduced or abolished ENaC activity in PHA patients, explaining the disease symptoms. (*J Clin Endocrinol Metab* 87: 3344–3350, 2002)

THE MINERALOCORTICOID hormone aldosterone regulates blood electrolyte levels by stimulating the reabsorption of sodium in epithelial cells in the renal tubule, respiratory and gastrointestinal tracts, and sweat and salivary glands (1–5). This effect appears to be mediated mainly via an increase in the activity of amiloride-sensitive epithelial sodium channel (ENaC) subunits (1–5). ENaC is located on the apical membrane of epithelial cells and transports Na^+ from the lumen into the cell (1–5). The reabsorbed sodium is then extruded from the epithelial cell back into the bloodstream by the Na/K-adenosine triphosphatase located at the opposite basolateral membrane of the cell. The reabsorption of sodium by ENaC is accompanied by an osmotic uptake of water to maintain a constant extracellular Na^+ concentration. Thus, ENaC plays important roles in electrolyte homeostasis and blood volume and blood pressure regulation (1, 2).

ENaC represents a special type of ion channel that is composed of a multimeric complex of subunits. In epithelial cells three different subunits, named α, β, and γ, have been identified (1–3, 6). The α-subunit is encoded on chromosome 12p13.1, whereas the β- and γ-subunits are encoded on chromosome 16p12.2–13.11 (7, 8). Sequence identity between β- and γ-subunit protein sequences is 32%, whereas identity between α- and β- or γ-subunits is 26–28% (9). The genes of these subunits show nearly identical intron-exon organization (9).

Lack of responsiveness to aldosterone, pseudohypoaldosteronism (PHA), was shown to include two distinct entities with different clinical and genetic characteristics: a mild renal form with autosomal dominant inheritance, and a severe multisystem form with recessive inheritance (10, 11). Almost all of previously described cases of PHA (12–18) can be classified into one of these two distinct forms (10). The multisystem form was shown by us and others (19–24) to emanate from loss of function mutations in ENaC subunits. In contrast, mutations that result in enhanced activity of ENaC cause a severe hereditary hypertension called Liddle's syndrome (25, 26). The involvement of ENaC in this syndrome, led to the suggestion that polymorphisms in ENaC sequence may be associated with hereditary forms of hypertension (27).

In the present study we examined four probands with multisystem PHA and identified novel mutations in the genes encoding the α- and β-subunits of ENaC. In our search for mutations we also determined the complete coding sequences of each of the three genes encoding α-, β-, and γ-subunits in individuals representing different ethnic groups.

Abbreviations: ENaC, Amiloride-sensitive epithelial sodium channel; PHA, pseudohypoaldosteronism; SNP, single nucleotide polymorphism.

Subjects and Methods

Subjects

Four families with multisystem PHA were examined in this study. In three families the parents were consanguineous (first cousins). Four affected infants (probands) presented in early infancy at 7–8 d of age with signs and symptoms of multisystem PHA (10). In these infants the diagnosis was based on clinical and laboratory characteristics of the disease, including severe dehydration, hyponatremia in the face of urinary salt losing (urinary sodium, >40 mmol/liter), hyperkalemia, and markedly increased serum aldosterone levels (Table 1). Their sweat and salivary Na^+ concentrations were very high, showing that salt loss is not limited to renal tubules, but is also observed in other epithelial cells responsive to aldosterone (Table 1). Initially all patients received iv fluid therapy with large amounts of 0.9% saline and ion exchange resin (Kayexalate, Sanofi-SyntheLabo, New York, NY) to decrease extremely high potassium levels.

Patient 11

This patient required 180 mmol/d sodium supplementation (NaCl and sodium bicarbonate) to remain in balance. He did not respond to fludrocortisone therapy. At the age of 15 yr he was still dependent on high amounts of sodium supplementation. His parents are first cousins of Indian Muslim origin.

Patient 13

In this patient long-term follow-up data were not available.

Patient 14

In addition to severe dehydration this patient presented with cardiac dysrhythmia due to hyperkalemia. During the first 6 wk of life he developed several episodes of severe salt-wasting crisis (sodium, <120 mmol/liter; potassium, 8.5–10.5 mmol/liter) despite treatment with iv saline and ion exchange resin. Eventually a percutaneous gastrostomy tube was inserted to administer high amounts of sodium supplementation (30–50 mmol/kg·d) as well as adequate nutrition. Treatment with high doses of fludrocortisone (0.3 mg/d) was ineffective. He had bouts of severe electrolyte imbalance even beyond the neonatal period, necessitating recurrent admissions. The frequency of these episodes decreased with age, and his growth and development were normal (height and weight along the 10th to 25th percentiles). At the age of 6.5 yr he died after cardiac arrest at home. The parents, who are of Scottish origin, had two additional children. The older sibling, a 2-yr-old girl, is affected as well. She had a similar clinical course and is presently thriving on sodium chloride and bicarbonate supplements. The youngest child is unaffected.

Patient 16

This patient also required high doses of sodium supplements during the neonatal period to remain in balance. At the age of 4 yr he still needs 150–180 mmol sodium/d and 15 g Kayexalate/d. On this regimen his condition is stable. The parents of patient 16, Pakistani Muslims in origin, were not known to be consanguineous, and their DNA samples were not available for analysis.

Other subjects

In addition to the above patients we determined the sequences of the subunits in 3 additional subjects: IH (a healthy adult Sephardic Jewish origin), 23 (an infant of English origin), and 103 (Arabic origin).

DNA isolation and marker typing

Genomic DNA was extracted from white blood cells as described by Miller *et al.* (28). The families were marker typed as previously described (8). Microsatellite markers specific to the chromosome 12p and 16p regions were selected as previously described (8). Genomic DNA was then amplified using these primers by PCR. The PCR reactions were carried out in 30 μl containing 50 mM KCl, 2.0 mM $MgCl_2$, 10 mM Tris-HCl (pH 8.8), 0.08% Nonidet P-40, 200 μM deoxy-GTP, 200 μM deoxy-TTP, 200 μM deoxy-CTP, 150 μM deoxy-ATP, 0.5–1.5 μCi [α-^{33}P]deoxy-ATP (3000 Ci/mmol or 92.7 TBq/mmol; Amersham Pharmacia Biotech, Arlington Heights, IL); 0.5–0.8 U Taq polymerase (recombinant); 0.25–0.5 μM of each primer; and 100 ng genomic DNA. After an initial denaturation of 94 C for 4 min, PCR was conducted for 30 cycles with denaturation at 94 C for 45 sec, annealing at 47–55 C for 45 sec, and extension at 72 C for 45 sec. For primer set D12S374, "touch down" PCR was performed with annealing at 54 C in the first PCR cycle, decreasing 0.5 C at every subsequent cycle for a total of 11 cycles. After this, 19 additional PCR cycles were performed at a constant annealing temperature of 55 C using the standard protocol described above.

For genotype analysis, PCR products were denatured in 10 μl formamide by heating to 94 C for 5 min and then electrophoresed on standard DNA sequencing gels and autoradiographed. All genotypes were scored independently by two investigators, and homozygosity regions were analyzed carefully.

PCR

The coding regions of α, β, and γ ENaC subunits along with partial or complete introns were amplified by PCR using genomic DNA as template. Based on the genomic and mRNA sequences of the subunits (7, 9, 19, 29–32), PCR primer sequences were selected in introns or in exon/intron junctions. Primer sets are described in Table 2. PCR reactions were carried out as previously described (9).

DNA sequencing

DNA sequences were determined by the dideoxy chain termination method using the ThermoSequenase-radiolabeled Terminator Cycle Sequencing kit and [α-^{33}P]dideoxy-NTPs (1500 Ci/mmol or 55.5 TBq/mmol; Amersham Pharmacia Biotech), following the standard protocol supplied by the manufacturer. All other conditions were as previously described (9). Some PCR products were also subjected to direct DNA sequence analysis using an ABI 373 automated DNA sequencer employing dye terminators, following a standard protocol by the manufacturer (PE Applied Biosystems, Inc., Foster City, CA).

The complete protein coding sequences of the ENaC subunit genes were determined for the following individuals: subunit α, 11, 16, and 103; subunit β, IH, 13, 14, and 23; and subunit γ, IH, 13, 14, 16, 23, and 103 (except γ exon 2).

Results

In this study we determined the complete protein-coding sequences of the α, β, and γ ENaC subunit genes, by sequencing genomic DNAs of PHA patients, their relatives, and normal individuals. The γ-subunit gene sequence provides the first complete coding sequence for this gene and is available under accession numbers AF356493 through

TABLE 1. Patients with multisystem PHA examined in this study

Patients	Nationality	Consanguinity	Aldosterone (pmol/liter)	Serum Na (mmol/liter)	Sweat Na (mmol/liter)	Serum K (mmol/liter)
11	Indian Muslim	Yes	>3,900	116	129–215	10.0
13	Syrian	Yes	51,600	125	136	8.8
14	Scottish	Yes	>40,000	118	152	10.2
16	Pakistani	No	>8,300	122	170	>12
Normal 0–3 months			<3,050	136–146	<50	3.5–5

AF356502. The sequencing results revealed the following mutations in the PHA patients examined (Table 3).

PHA patient 16

Sequencing of the genomic DNA of PHA patient 16 showed an insertion of a T in exon 8 of α ENaC at position 1439 of the mRNA sequence (X76180; Fig. 1). This mutation changes the Tyr^{447} codon sequence from TAC to TTAC, which leads to a premature stop codon at K459.

PHA patient 11

Sequencing of the genomic DNA of patient 11 revealed a homozygous mutation in exon 11 of the α ENaC gene at a position corresponding to nucleotide 1621 in mRNA sequence X76180 (Fig. 2). The observed change of C to T in this position changes the Arg^{508} codon to a premature stop codon. Parents who are first cousins showed heterozygosity for this mutation (Fig. 2). This confirms the hereditary origin of the mutation in patient 11.

TABLE 2. Primer sets and PCR conditions that were used to amplify exons and introns of the human α, β, and γ ENaC genes

Primer set	Exon	Forward	Reverse	Temperature (C)	$MgCl_2$ (mM)	Product (bp)
A1F–2R	2	acccttgctctctccaatccac	ggggcagagggactaaccgac	55	6	527
A3aF–3R	3	aggtacccggaaattaaagagg	ggaccctcaggcgctgcaag	55	3.5, 6	166
A3F–4R	3	agctccttcaccactctcgtg	gtcaggaaaggagcggagcccatg	60	2	277
A5F–5R	4	cctctgactctagtctctgtgtc	ggagccaggcaggactgactc	55	1.5	240
A6F–7R	5, 6	gaccctactctcttttcctg	ccttccaggcctcccagtcag	55	4.5	666
A8aF–8R	7	gatctcttggtgtatgtggg	cacggaatcaggttgggcctc	55	3.5, 6	149
A9F–9R	8	cctctccaccctcctcccttc	ggggctccctggagtctcac	60	2	162
A10F–11R	9, 10	acaggcatctctctgtacccac	acccatcccttccccacactc	60	2	290
A12F–13R	11, 12	gaccttgatgacacccccattc	caggctccatccaggcacgac	60	2	333
A14F–16R	13	agaaccctctgtcccatcgtc	gccttggtgtgagaaacctctc	55	4.5	443
B15F–16R	13	gctggtggccttggccaagag	agggtaggaaccaggtgaag	60	1.5, 2	502
G1.2F–1.3R	2	atgcgaagttcctaagcc	ctttgatcttctctccgg	55	4	215
G1F–1.1R	2	gtcccatcctcgccatg	gccagtgtgtcaactttc	50	4	416
G2F–2R	3	ccctctccctgacttttcctc	aatgagaaggtgaaatcttacc	60	1.5, 6	356
G3F–3R	4	cgcatctcctcttattcacag	agagcagcattctCtcctgac	60	1.5, 2.5	233
G4F–4R	5	gacccattttcttcctccatag	ccttggcacaggtttccttac	60	6	147
G5F–5R	6	caggtGgtcttatcctcccag	ctccaagcctatggaaatgag	60	6	219
G6F–6R	7	gaggacagggctgagtgtgtg	cagggctgggtgccccTgcca	60	1.5, 6	165
G7F–7R	8	tcctgggtctcctctttcaga	ctggagctgggtctGactcac	60	1.5, 6	159
G8F–9R	9, 10, 11	gccctctcccttgtccctcag	gaacagggtagaggtaacttac	56	1.5	528
G9F–9R	11	cgctttctctctccgttgtag	gaacagggtagaggtaacttac	56	1.5	105
G10F–10R	12	ttcacctgttggaattttgcag	gaaggaagccactctactcac	60	2.5, 6	119
G11F–11R	13	ttgatggtgtggcttggcctg	tacggggagcttctggacatg	60	6	229
G12F–12R	13	gcagaaagccaaggagtggtg	gatctgtcttctcaaccctgc	60	6	275

The nucleotides marked in *capital letters* are based on previously designed primer sequences (19) and do not match the genomic DNA (gDNA) sequence: G3R (C → t in gDNA), G5F (G → t in gDNA), G6R (T → c in gDNA), and G7R (G → c in gDNA).

TABLE 3. Mutations identified in ENaC subunits in patients with autosomal recessive multisystem PHA

Ethnicity	No. of families	Subunit	Location	Mutation	Codon change	Het.	Reference
Northern Europe	1	α	Exon 2	256 C→T	Arg53stop[a]		20
Saudi	3	α	Exon 2	302delTC	Ile68fr	F, M	19
Hispanic	1	α	Exon 3	604delAC	Thr169fr		20
			Exon 8	1404delC	Phe435fr		
Swedish	1	α	Exon 4	828delA[a]	Ser243fr	F	22
			Exon 8	1449delC	His450fr		
Pakistani (16)	1	α	Exon 8	1439insT	Tyr447fr	ND	This study
Swedish	1	α	Exon 8	1449delC	His450fr	F, M	22
Indian Muslim (11)	1	α	Exon 11	1621C→T	Arg508stop	F, M	This study
Iranian-Jewish	2	α	Exon 11	1621C→T	Arg508stop	F, M	19, 20
Swedish	1	α	Exon 13	1784C→T[a]	Ser562Leu	F	22
			Exon 8	1449delC	His450fr		
Arab	1	β	Exon 2	236G→A	Gly37Ser	F, M	19
Ashkenazi-Jewish	1	β	Exon 3	647insA	Leu174fr		20
			Exon 5	915delC	Ser263fr		
Scottish (14)	1	β	Intron 12	1669+1 G→A	Abn. splicing	F, M	This study
Indian	3	γ	Intron 2	318-1 G→A	Abn. splicing	F, M	23
Japanese	1	γ	Intron 12	1570insGA	Abn. splicing	F, M	21
			Exon 13[a]	1627delG	Val543fr		

For the α- and β-subunits the nucleotide numbers are based on mRNA sequences X76180 and X87159, respectively. Het., Demonstrated heterozygosity in parents' DNA (F, Father; M, mother). Patients with a single entry in the *Mutation column* are homozygous for that mutation. The other patients show compound heterozygosity as indicated. ND, Not determined.

[a] The numbers marked have been corrected from the original report.

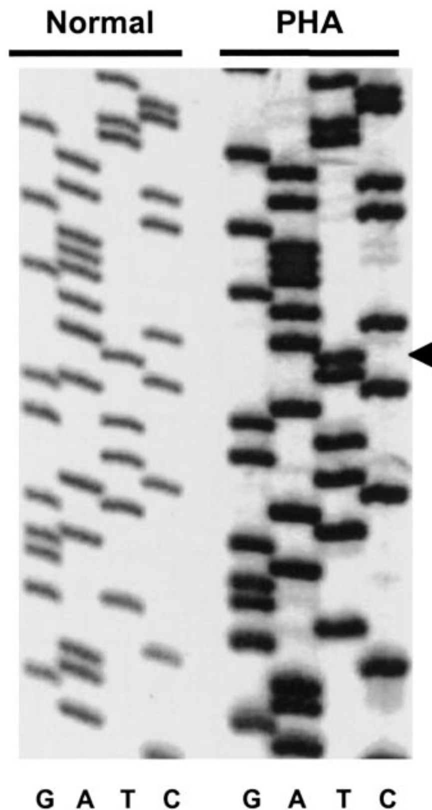

FIG. 1. Sequence of the region of α ENaC exon 8 that shows a mutation in PHA patient 16. Sequencing was performed using primer A9F. The identified mutation is an insertion of t (marked by *arrowhead*) at nucleotide1439 in exon 8 leading to a Y447frameshift and K459stop. Normal, AGAACGTGGAGTACTGTGACTACAGAAAGCACAG; PHA 16, AGAACGTGGAGTACTGTGACTtACAGAAAGCACAG (mutation *underlined*).

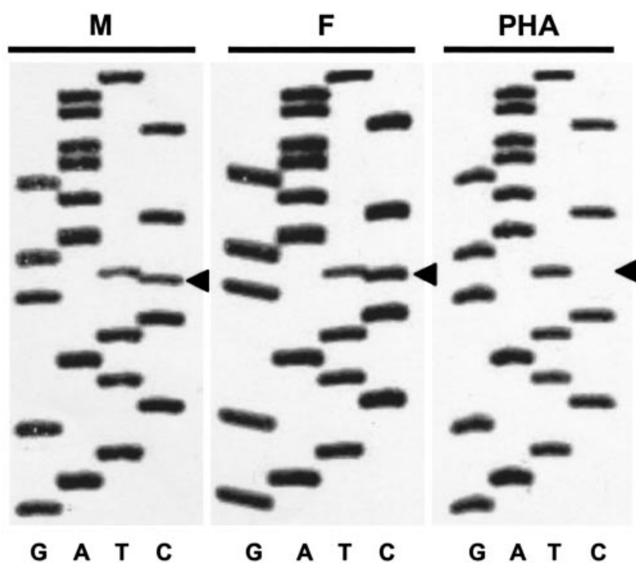

FIG. 2. Sequence of the region of α ENaC gene exon 11 that shows a mutation in family 11. Sequencing was performed using primer A12F. M, Mother; F, father; PHA, son. Heterozygosity of the parents can be clearly seen, with a T and a C at the same location, whereas the sequence of their PHA son shows only a T (marked by *arrowheads*). Sequence shown: normal, GATGCTATCGCGACAGAACAAT; PHA 11, GATGCTATCGtGACAGAACAAT (mutation *underlined*).

PHA patient 13

Linkage analysis for this patient showed evidence of linkage to 16p12.2–13.11 encoding the β and γ ENaC subunits (8). Yet, sequencing of the protein-coding region of these two genes did not reveal a mutation. Thus, a mutation in a regulatory region or another relevant functional site may be responsible for this case.

PHA patient 14

Sequencing of the genomic DNA of patient 14 revealed a homozygous mutation of G to A in the first nucleotide at the 5′-splice site of intron 12 (Fig. 3). The parents were heterozygous for this mutation, confirming the hereditary origin of the mutation in the patient.

Sequence variants in genes encoding ENaC subunits

Table 4 presents a list of all of the sequence variants observed in all three genes encoding α, β, and γ ENaC subunits. In the α-subunit gene we identified only one variant that changes an amino acid, Thr^{663}, to Ala. In the β-subunit gene we observed only two variants: one altering, Gly^{314} to Ala, and a silent change at the 1174th position in mRNA leaving amino acid sequence unchanged (Table 4). The differences observed in the γ-subunit gene are elaborated upon in *Discussion*.

Discussion

In this study we have examined four families with autosomal recessive multisystem PHA. All index cases showed the typical clinical characteristics of multisystem PHA, as described previously (10). In three patients we have identified the following mutations in α- and β-subunits of ENaC

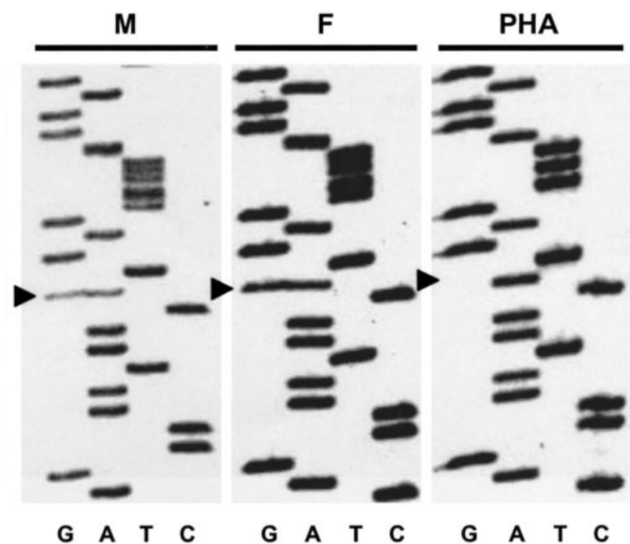

FIG. 3. Sequence of the β ENaC gene exon 12/intron 12 junction that shows a mutation in family 14. Sequencing was performed using primer B12F. M, Mother; F, father; PHA, son. Heterozygosity of the parents can be clearly seen, with a G and A at the same location, whereas the sequence of their PHA son shows only an A (marked by *arrowheads*). Sequence shown: 3′ of exon 12; 5′-end of intron 12; normal, GCCAATAAC gtgagttt (encoding AlaAsnAsn); PHA 14, GCCAATAAC atgagttt (mutation *underlined*).

TABLE 4. Sequence variants observed in the α, β, and γ ENaC subunit genes that are not associated with PHA

Subunit	Location	Nucleotide or codon change	A.A. change	Frequency[a]	Additional references
Alpha	Exon 2 (UTR)	713ins g		5/5	
	Intron 4	t997a		3/3	
	Intron 4	g1266t or del			33
	Intron 4	1657ins t		3/3	
	Intron 5	g1926del		3/3	
	Intron 5	t1990g		3/3	
	Exon 13	a2086g ACC-GCC	T663A	1/3 (103)	33, 34
Beta	Exon 6	g1068c GCG-CCG	G314A	4/4	9
	Exon 7	t1174c GAT-GAC		4/4	
Gamma	Intron 1	g147c		1/3 (23)	
	Exon 2 (UTR)	g164a		1/3 (23)	
	Exon 2 (UTR)	a181del		3/3	
	Exon 6	c1016t TCC-TTC	S339F	6/6	
	Exon 6	a1048g ACA-GCA	T350A	6/6	
	Exon 7	c1106a TCC-TAC	S369Y	6/6	
	Exon 7	g1124a GGC-GAC	G375D	6/6	
	Intron 8	c135del		6/6	
	Exon 9	c1372a CGC-AGC	R458S	6/6	
	Exon 13	c1596t GGC-GGT		6/6	
	Exon 13	t1599c GGT-GGC		6/6	

UTR, Untranslated region. The nucleotide on the *left* is based on the standard sequence, and the nucleotide on the *right* indicates the finding in this study or other cited references. The numbering used for the exons follows the standard mRNA sequences. The numbering for the introns and other UTRs follows the genomic DNA (gDNA) sequences. The following sequences were taken as standard references: alpha: Z92978 (gDNA), Z92980 (gDNA), Z92981 (gDNA), X76180 (mRNA); beta: X87159 (mRNA); gamma: U53836 (gDNA), U53850 (gDNA), X87160 (Voilley mRNA).

[a] Frequency refers to the number of independent genes from different individuals sequenced in this study.

that explain the molecular basis of their multisystem PHA syndrome.

Insertion of a T in exon 8 of the α ENaC gene

This novel mutation was detected in Muslim Pakistani PHA patient 16. The mutated gene encodes a truncated α ENaC subunit and thus would unequivocally explain the observation that the patient suffered from multisystem PHA.

R508stop mutation in exon 11 of the α ENaC gene

This homozygous mutation was detected in patient 11, at a position corresponding to nucleotide 1621 in mRNA sequence X76180. Parents of the patient, who are first cousins of Indian Muslim origin, showed heterozygosity for this mutation. In our earlier studies we observed this mutation of Arg^{508}stop in two families of Iranian Jewish origin (20). The mutation is located at a CpG dinucleotide (Fig. 2) that has been observed as a mutation hot spot in many genes. Thus, it is possible that the same mutation arose independently in an Indian Muslim family as well.

Splice site mutation in intron 12 of the β ENaC gene (5′ss g→a)

This novel homozygous mutation was detected in the genomic DNA of patient 14, who was of Scottish origin. The mutation of G to A in the first nucleotide at the 5′-splice site of intron 12 changes the conserved GT sequence at the 5′-end of introns, preventing correct splicing of the mRNA. This mutation would thus result in the absence of a functional β ENaC subunit and explains the molecular basis of the multisystem PHA observed in this patient.

General features of mutations in autosomal recessive multisystem PHA

Examination of all of the known autosomal recessive multisystem PHA patients shows the following features. 1) All cases deciphered show mutations in both alleles encoding one of the subunits of ENaC. The majority show homozygous mutations, with both parents displaying heterozygosity for the mutations, and others are compound heterozygotes. 2) The mutations may be observed in any of the three subunits of ENaC. 3) The mutations observed include single nucleotide changes, deletions, insertions, and splice site junction mutations leading to the production of an inactive protein. 4) With one exception, different mutations are observed in different ethnic groups. 5) Most of the mutations appear in the α-subunit, consistent with an important role for this subunit in ENaC function (24). 6) The mutations have helped define functional domains of the subunits (for review, see Ref. 24). 7) In contrast to Liddle's syndrome (24–26), none of

the mutations in multisystem PHA appear in the carboxyl-terminal region.

Sequence variants in genes encoding ENaC subunits

The question of polymorphisms in ENaC subunits has recently received extensive attention because of possible association with some hereditary forms of hypertension (20). In Table 4 we present the list of all single nucleotide variants observed in all three genes encoding ENaC α-, β-, and γ-subunits. Only one variant we observed in the α-subunit gene changes the Thr^{663} codon to Ala. This variant has been previously reported in different individuals (29, 32–34), indicating that it represents a genuine single nucleotide polymorphism (SNP). Yet, its biochemical significance remains unknown. In the α-subunit gene we have identified additional sequence differences in the untranslated region of exon 2 and in the introns of the gene compared with previous reports. As some of these differences were observed in all of our subjects from different ethnic groups, it is possible that previous sequences based on a single report may contain sequencing errors at some of these bases.

In the β-subunit gene we observed only two different single nucleotide changes. The functional significance, if any, of the amino acid change remains to be determined. Several previous studies examined partial sequences of β- and γ-subunits (only selected exons in the carboxyl-terminal region) (35–37) in hypertensive subjects. Although Persu *et al.* (35) identified novel rare SNPs, no mutation was found to be definitively associated with hypertension (35–37). Exons 8 and 12, noted in a previous study (35), are here numbered 9 and 13, as we now know that there are 13 exons in the β ENaC gene (9), not 12.

We sequenced the complete coding sequence of the γ ENaC gene (GenBank accession no. AF356502) from six individuals from different ethnic origins, to resolve the discrepancies in the previously published γ ENaC complete mRNA sequences (7, 32) and partial genomic sequences (30). In our subjects we observed complete conservation of the coding sequence. The single nucleotide change we observed was located in the 5′-UTR (Table 4). Yet, we detected eight single nucleotide differences, five of which also change the amino acid sequence compared with previously published sequences (Table 4). Because of the discrepancies among previously published mRNA and genomic sequences themselves, it is likely that some or all of the differences we observed may reflect sequencing errors, rather than genuine SNP. As we sequenced six individuals from different groups, we suggest that the amino acid sequence based on our genomic sequencing be used as the standard γ ENaC sequence rather than the previously published sequences with the differences listed in Table 4.

In summary, our sequencing of multisystem PHA patients revealed novel homozygous mutations in the α and β ENaC genes that should result in reduced or abolished ENaC activity. Consistent with the autosomal recessive inheritance of the disease, the parents of at least two of the patients were heterozygous for the mutation. The present results revealed only a few sequence variants among individuals from different ethnic groups, indicating that the gene and the coding sequences of the ENaC subunits are very strongly conserved.

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