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Original Article

A novel tyrosine 516 stop mutation in the α subunit of the epithelial sodium channel causing Pseudohypoaldosteronism in the affected offspring and A subclinical, salt-losing phenotype in a heterozygous mother

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طفرة غير مألوفة في وحيدة الفا من قنوات الصوديوم الطلائية مسببة لمرض الالدوستيرون الكاذب عند ام

د احمد الزهراني –استشاري الاطفال –مستشفي النور التخصصي- مكة المكرمة

الملخص العربي: :

المقدمة والخلفية : يعتبر النوع الأول من نقص الالدوستيرون الكاذب مرض نادر الحدوث يتميز بفقد شديد ومبكر للاملاح وارتفاع في بوتاسيوم الدم وحموضة دموية و مقاومة لعلاج المنيرالوكورتيكويد . يسبب هذا المرض تحول جيني متماثل او متنافر اللواقح في اي من الوحيدات الثلاث بقنوات الصوديوم الغشائية.

الحالات والمناقشة: هذه الدراسة عبارة عن تقرير لعائلة لديهم اربعة اطفال متأثرين بهذا المرض تم وصف حالتهم السريرية والكيميائية الحيوية والتحليل الجيني الذي اثبت وجود تحول جيني لم يتم كشفه من قبل في وحيدة الفا من قنوات الصوديوم الطلائية وذلك عند الحمض النووي 516 مستبدلا ترتيب ادت الى تحويل البروتين من 721 حمض نووي الى TAG بالاحماض النووية TAC الاحماض النووية . 516 حمض نووي مما يؤدي الى فقد وظيفة قنوات الصوديوم الطلائية وظهور المرض
ومع ان الام لاتعاني من المرض الا ان تحاليل الدم اثبتت ارتفاع الالدوستيرون وارتفاع نسبة الصوديوم في البول ربما فقد بسيط في الاملاح عند الام في طفولتها المبكرة لم يتم ملاحظته طبيا ، الاب حامل للمرض ولم يكن يعاني من أي اعراض وتحاليله كانت سليمة.

الخلاصة: طبقا لمعلوماتنا هذه الحاله الرابعة والعشرون المسجلة عالميا و الخامسة عربيا لمثل هذه الحالات مصابة بخلل في هذا الجين وتمثل تحولاجينيا حديثا ومكتشفا في هذه النقطة الجينية، واول تقرير عن ارتفاع الالدوستيرون وارتفاع الصوديوم اليومي في شخص ناقل للمرض.

الكلمات الدالة: الالدوستيرون الكاذب - المنيرالوكورتيكويد- التحول الجيني – اللواقح الحمض النووي

ABSTRACT

Rationale and Background:: Pseudohypoaldosteronism type I is a rare life-threatening condition characterized by Severe, early-onset salt wasting, hyperkalemia, metabolic acidosis and resistance to Mineralocorticoids. The condition is inherited in either an autosomal recessive or dominant manner. Autosomal recessive Pseudohypoaldosteronism type I is a multisystem, severe, life-long condition, caused by homozygous or compound heterozygous mutations in any of the three subunits, (α, β, or γ), of the Epithelial sodium channel.

The cases and discussion:

I reported a family with four affected siblings with autosomal recessive or multi-system Pseudohypoaldosteronism type I.,their clinical picture and biochemical abnormalities described. Genetic mutation analysis revealed a novel nonsense mutation in the alpha subunit of ENaC, at amino acid 516, substituting a **TAC [Y]** for a **TAG [STOP].** This truncated the protein, shortening it from 721 amino acids to 516 amino acids, leading to loss of channel activity and resulting in disease. Although asymptomatic, analysis of the laboratory values of the heterozygous mother revealed high serum aldosterone with high urinary sodium but normal clinical and biochemical analysis in the heterozygous father. However, a mild salt loss might have been missed in the infantile period.

Conclusion:This is the first report of a high serum aldosterone level with high urinary Na in a heterozygous carrier, and represented a novel mutation in this gene.

***Keywords*: Pseudohypoaldosteronism- mutation-DNA**

INTRODUCTION

Type I pseudohypoaldosteronism (PHA1) is a rare salt-wasting condition manifested soon after birth by vomiting, lethargy, severe dehydration, hyponatremia, hyperkalemia, metabolic acidosis with high plasma aldosterone and renin concentrations. [1,2,]

Two types of PHA1 have been described; the renal or autosomal dominant type due to aldosterone receptor defects, and the multisystem or autosomal recessive type, a severe, life long condition, caused by homozygous or compound heterozygous mutations in the subunits (α, β, or γ) of the Epithelial sodium channel (ENaC).[1,2,3]

The amiloride-sensitive epithelial sodium channel (ENaC) is a highly selective Na channel found at the apical membrane of salt-reabsorbing tight epithelia of tissues including the distal nephron, the distal colon, the salivary and sweat gland, and the lungs.[2] In these polarized epithelia, the ENaC mediated entry of sodium into the cell represents the rate-limiting step for vectorial movement of sodium from the mucosal to the serosal side. In the kidney, ENaC activity is controlled by aldosterone, serving to maintain salt homeostasis and blood pressure.[2, 3] and shares 35% identity in their amino acid sequences. [4,5] Each subunit has two transmembrane domains with short cytoplasmic N- and C- termini, and a large extra cellular

loop.[4,5,6,7] The human ENaC genes have been cloned, and several genetic mutations in the coding regions of the alpha, beta and gamma subunits of ENaC have been described recently.[8,9,10,14,16,18]

Herein, we describe the clinical and biochemical presentations, and a novel mutation, in the alpha subunit of ENaC, in a consanguineous family with four affected children.

Cases and Report

The subjects are four siblings who are the products of first-degree consanguineous Yamane parents. The mother, 29 years old and the father, 31 years old, are both asymptomatic and healthy.

Laboratory values showed serum aldosterone was normal in the father, 13ng/dl. All four siblings had the classic symptoms of PHAI with no respiratory manifestations. Three of the children died of complications from the disease. The third child is living at 3 years old. Table 1.

Case I

The first baby, a male, was brought to a hospital on the third day of life with vomiting and refusal to feed. He was admitted with severe dehydration, hyponatremia and hyperkalemia, and died two days later. No detailed information is available.

Case II

The second baby, a girl, born on 20/11/05, was brought to our hospital at 6 days old with vomiting, irritability and refusal to feed. She was found to be dehydrated with severe hyponatremia, hyperkalemia, and metabolic acidosis. System examination upon admission was unremarkable, with normal female external genitalia and no respiratory symptoms. Her serum aldosterone was elevated at 4200 ng/dl, (N <35.5ng/dl), PRA was 453 pg/ml/h, (N < 33 ng/ml/h), and was resistant to high dose fludrocortisone, (0.3mg /d). The patient was treated for PHAI with a large amount of sodium chloride, IV and PO, and Kayexalate. However, she developed severe hyperkalemic arrhythmia and died on 27/1/06 at 2 months old.

Case III

The third baby, a girl, was born on 23-6-2007 with a birth weight of 3 kg. She presented to the ER on the fourth day of life with vomiting, poor feeding, irritability and severe dehydration. Her blood pressure was 70/40; weight 2.7 kg with a normal system examination and normal female external genitalia.

Laboratory values showed persistent hyponatremia, severe hyperkalemia and metabolic acidosis, unresponsive to high dose fludrocortisone, 0.3mg /d(three tablets daily), and requiring large amounts of NaCl. Her serum aldosterone was elevated at 781 ng/dl N <35.5ng/dl. PRA of 381 pg/ml/h, (N<33 ng/ml/h), was also high, with normal serum cortisol at 25 ug/dl and 17-OH progesterone at 1.4 ug/ml. The patient was hospitalized for one year in our hospital due to frequent severe electrolytes disturbances needs frequent resuscitations. She had no respiratory symptoms during admission. She was discharged on 3% sodium chloride,

40cc PO every 4 hours, (about 7 gm/day), and Kayexalate, 7gm q 6 hourly. On follow up, she is thriving well, on these doses, with normal electrolytes.

Case IV

The fourth child, a baby boy, was born on 6-06-2009 with a birth weight of 3.5 kg. He was kept in our hospital as we were anticipating the condition. In the first few days, he was showing a picture of hyponatremia, hyperkalemia, metabolic acidosis, and resistance to high dose fludrocortisone 0.3mg /d. He was started on a 3% sodium chloride solution and Kayexalate rectal enema. His aldosterone was elevated at 137 ng/dl and PRA was high at 312 pg/ml/h. He had a stormy course in the hospital with frequent and severe episodes of hyperkalemia and hyponatremia, but no respiratory distress or chest infections. The last episode was at four months old when he developed severe hyperkalemia with serum potassium of 12 mmol/L. Cardiac arrhythmia and resistance to intensive measures complicated this episode. He arrested and died on 28-10-09 at almost four months old.

Mother

She was 29 years old healthy, asymptomatic lady, no history of neonatal admission, chest infection or significant illness, no history of polyurea or polydypsia or any other symptoms.
Her urinary sodium was elevated 185mmol/L (N <40 mmol/L) and her serum aldosterone of 93.5 ng/dl (Normal <35.5ng/dl).

Father

He was 31 years old with no clinical or biochemical abnormality including serum Na of 141 nmol/L ,K 4.1, serum aldosterone 13ng/dl , urinary Na 27 nmol/L.
No other family history of similar condition or early deaths

Genetic Mutation analysis

Blood samples for molecular genetic analyses were taken, after informed consent, from both parents and two siblings Cases III and IV and sent to the Genetic department at Yale University,USA, were Genomic DNA was extracted from peripheral blood leucocytes, and the subunits of the human amiloride-sensitive sodium channel, (ENaC), SCNN1A, SCNN1B and SCNN1G, were amplified using primers encompassing the coding regions and the flanking intronic sequences, as described previously. [8] The nucleotide sequences of both strands of the PCR products were directly determined using an automated fluorescent sequencer (ABI Prism 310 Genetic Analyzer, Perkin-Elmer Corp, Wellesley, MA). The PCR products were analyzed by agarose gel electrophoresis.

Table1: Patient's characteristics & lab results

Family	Presented	Age	Aldosteron	PRA	Serum Na	Serum	DNA	U Na
Case I	3ed day	Live5days	-	-	115	8.9	-	-
Case II	6th day	Live 2 mo	4200	453	117	9.5	-	-
Case III	4th day	A live	781	381	121	10.5	Y516 TER Homozygous	154
Case VI	4th day	Live 4 mo	137	312	124	12	Y516 TER Homozygous	195
father	31 yr	A live	13	-	141	4.1	Y516 TER Homozygous	27
mother	29 yr	A live	93.5	-	137	4.3	Y516 TER Homozygous	185

Aldosteron N <35.5ng/dl , PRA N < 33 pg/ml/h , U Na N <40

RESULTS

The clinical syndrome manifested in these siblings by salt wasting, hyperkalemia, and metabolic acidosis associated with elevated plasma renin activity and aldosterone levels is characteristic of PHAI. The phenotype arises from loss of function of the epithelial sodium channel. It is caused by mutations in any of the three subunits of ENaC, as an autosomal recessive disease or with mutations in the Mineralocorticoid Receptor (MR), as an autosomal dominant disease. [1, 2, 3]

We have demonstrated that the cause of PHA1 in our family is a nonsense mutation in the alpha subunit of the epithelial sodium channel, occurring at amino acid 516. This substitutes a TAC [Y]for a TAG [STOP], changing a Tyrosine to a Stop Codon. This truncates the SCNN1A protein at amino acid 516, shortening the protein from 721 amino acids to 516 amino acids. This truncation eliminates the second Transmembrane Domain and the PPXY domain of the protein. The second transmembrane domain is implicated in having a role in conferring ion selectivity to the protein, and in contributing to its conduction pore. The critical PPXY Domain has been shown to be the site of interaction with the E3 ubiquitin ligase, Nedd4-2. These are domains proven to be critical for functioning of the alpha ENaC subunit. The homozygous mutation confers affected status in the two children and the heterozygous mutation confers carrier status in both parents. We also observed that our patients did not have respiratory distress or get chest infections like most of the reported cases of PHA1. [11, 12, 13]

Surprisingly, the mother who is an asymptomatic heterozygous carrier demonstrated high serum aldosterone and high urinary sodium, a finding not reported before.

Table 2: ENaC subunit genes mutations responsible for PHA1 reported worldwide.

Phenotype	Ethnici	Subunit	Location	Mutation	Codon	change Reference
severe	Yaman	Alpha	Exon 11		Tyr516stop	This study
severe	Yaman	Alpha	Exon 11		Tyr516stop	This study
severe	Somali	Alpha	Exon 13	c.1684T>C	S562P	Felix et al14
Mild*	Polish	Alpha	Exon 5	1078G→	Gly 327 Cys*	Edelheit et al9
Mild *	Swedish	Alpha	Exon 13	1784C→	Ser 562 Leu*	Schaedel et al15
?	Northe	Alpha	Exon 2	256 C→	Arg 563 stop	Kerem et al11
Severe	Saudi	Alpha	Exon 2	302 del TC	Ile 68 fr	Chang et al8
Severe	Hispanic	Alpha	Exon 3	604 del AC	Thr169fr	Kerem et al 11
Severe	Swedish	Alpha	Exon 4	828 del A	Ser243fr	Schaedel et al15
Severe	Hispan	Alpha	Exon 8	140 del C	Phe435fr	Kerem et al11
Severe	Pakistan	Alpha	Exon 8	1439 ins T	Tyr447fr	Saxena et al10
Mild*	Polish	Alpha	Exon 8	1449 del C	His450fr*	Edelheit et al 9
Mild/sever	Swedish	Alpha	Exon 8	1449 del C	His450fr	Schaedel et al15
NLT	Turkish	Alpha	Exon 8	1455 del C	Ser 452 fr	Edelheit et al9
Severe	Dutch?	Alpha	Exon10	Arg492stop	Bonny et al16	
Severe	Indian	Alpha	Exon11	162 C →	Arg 508 stop	Saxena et al10
Severe	Jewish	Alpha	Exon11	1621 C →	Arg508stop	Chang et al8
?	Arab	Beta	Exon 2	236 G →	Gly 37 Ser	Chang et al8
?	Jewish	Beta	Exon 3	647 insA	Leul 74 fr	Kerem et al11
?	Jewish	Beta	Exon 5	915 del C	Ser 263 fr	Kerem et al11
Severe	Arab	Beta	Intron	1669+1G→	Abnormal	Edelheit et al9
Severe	Scottish	Beta	Intron	1669+1G→	Abnormal	Saxena el at 10
NLT	Indian	Gamma	Intron 2	318 - 1G→	Abnormal	Strautnieks
Severe	Japanese	Gamma	Intron	1570 - 1G→	Abnormal	Adachi et al18
Severe	Japanese	Gamma	Exon 13	1627delG→	Val 543fr	Adachi et al18

DISCUSSION

In this study, we reported a consanguineous family with autosomal-recessive, multisystem PHA1. Within the first few days of life their children manifested severe, early onset salt-wasting, dehydration, hyperkalemia, hyponatremia, metabolic acidosis, high urinary sodium, hyperaldosteronism, hyperreninemia with normal adrenal function, normal cortisol and 17-

OH progesterone, without respiratory distress. We have identified a novel nonsense mutation in the alpha subunit of the epithelial sodium channel, (SCNN1A), at amino acid 516 that substitutes a TAC (Y) for a TAG (Stop). This changes a tyrosine to a stop codon, truncating the protein at amino acid 516, shortening it from 721 to 516 amino acids. This truncation eliminates the second transmembrane domain and the critical **PPXY** domain, domains known to be significant for functioning of the epithelial sodium channel subunits.

The two affected children Cases III and IV were homozygous for the mutation and both parents were heterozygous carriers, indicative of autosomal recessive inheritance. To our knowledge, this finding brings to 24 the number of reported independent mutations, known worldwide, in the coding regions of the three subunits of ENaC (Table 2). The majority, (20 out of 24), of the multisystem PHA1 associated mutations leads to abnormal length mRNA or protein because of deletions, insertions or splice site mutations in the DNA. These structural changes within the proteins lead to loss of function of ENaC and a severe type of PHA1, resulting in the inability of the subject to regulate volume changes.
Interestingly, 16 out of 24 of the reported mutations[9], are in the alpha subunit of ENaC. (Tab 2)
The characteristic features of genetic mutations in the autosomal recessive multisystem PHA1 patients are as follows: [10]

1- All examined cases showed mutations in both alleles encoding one of the subunits of ENaC. The majority shows homozygous mutations, with both parents displaying heterozygous mutations. The others are compound heterozygote.

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 changes leading to the production of an inactive protein.

4- Most of the mutations appear in the alpha subunit, consistent with an important role of this subunit in ENaC function.

5- The mutations have helped define functional domains of the subunits.

6- In contrast to Liddle's syndrome, resulting from gain-of-function mutations in the ENaC subunits, none of the mutations in multisystem PHA1 appears in the carboxy-terminal region.[10]

The fact that none of the four affected siblings with systemic PHA1 exhibited chest symptoms, like many of the reported cases, [11, 12, 13] could be due to phenotypic heterogeneity of the condition.

The mother was an asymptomatic heterozygous carrier but she had an elevated serum aldosterone level of 93.5 ng/dl (N<35.5 ng/dl), and a high urinary sodium. Although, asymptomatic, a mild salt losing phenotype could have been missed during infancy. These findings, in a carrier, have not been reported in the literature. However, high sweat sodium and chloride levels in a heterozygous carrier have been reported by Felix G. Riepe et al.[14] This may be due to phenotypic heterogeneity in systemic PHA1 or a dominant-negative effect

of the mutant allele. This suggests that one copy of the mutant gene confers a mutant phenotypic effect that is subtle and not as severe as the two- copy mutation within the gene.

Conclusion: our sequence of systemic PHA1 patients, in a consanguineous kindred, revealed a novel homozygous, nonsense mutation at amino acid 516 in the alpha subunit of ENaC. This truncates the protein, shortening it from 721 AA to 516 AA, leading to a decrease in epithelial sodium channel activity.

The parents are heterozygous carriers, consistent with the autosomal recessive inheritance of the disease. The presence of a high serum Aldosterone and high urine sodium in the heterozygous mother cannot be fully explained. However, it may be due to the phenotypic heterogeneity of the disease, or the dominant effect of the mutated allele, (Dominant-Negative effect theory).

Identification of the molecular basis of PHA1 is helpful for early diagnosis, understanding of the path physiology of the condition, genetic counseling and possible pre-implantation selection in affected families.

CONCLUSIONS

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