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Original Article

Hypokalaemia and paralysis in the Thai population

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Abstract

Background. Hypokalaemia with paralysis is a syndrome common in Thailand. This syndrome may result from hypokalaemic periodic paralysis (HypoPP), thyrotoxic periodic paralysis (TPP) or distal renal tubular acidosis (dRTA). We prospectively investigated the nature of this syndrome in afflicted Thai patients.

Methods. Blood and urine samples were collected from 47 patients during attacks for multiple biochemical and thyroid function tests. A long acid loading test was performed in all euthyroid patients. Mutation analyses were done in all HypoPP and TPP patients.

Results. Of the subjects, 34 completed the study. Of those, 11 (32%), eight (24%) and 15 (44%) had TPP, dRTA and HypoPP, respectively. Patients with dRTA and TPP were older than those with HypoPP. Males were more prevalent than females in HypoPP and TPP; the reverse was true for dRTA. Two-thirds of the HypoPP cases were sporadic. The majority of the HypoPP and dRTA patients resided in northeastern Thailand. Of the 11 TPP patients, nine (82%) had no previous thyroid disease. Moreover, four out of 11 patients (36%) had subtle clinical signs of hyperthyroidism; three of eight dRTA patients had renal stones, nephrocalcinosis or both. Only two patients had metabolic acidosis at the time of presentation. No common mutations were found in the HypoPP and TPP patients.

Conclusions. In most of our patients, HypoPP is sporadic and not associated with the common mutations reported previously. Clinical clues that can assist in differentiating between the causes of hypokalaemia and paralysis are age at onset, gender and geographic region residence of the patients. However, the absence of previous histories of thyroid disease or overt thyrotoxicosis, and of stone disease/nephrocalcinosis or metabolic acidosis does not exclude the diagnosis of TPP or dRTA.

Keywords: distal renal tubular acidosis (dRTA); hypokalaemia; hypokalaemic periodic paralysis (HypoPP); periodic paralysis; thyrotoxic periodic paralysis (TPP)

Introduction

The syndrome of hypokalaemia-induced paralysis is rare, and commonly is caused by diuretic or abnormally elevated levels of mineralocorticoid hormones. However, the causes of hypokalaemia with severe paralysis are different in Southeast Asia. This syndrome may result from heterogeneous disorders, including hypokalaemic periodic paralysis (HypoPP), thyrotoxic periodic paralysis (TPP) and distal renal tubular acidosis (dRTA). All of these disorders produce clinical syndromes of hypokalaemia with muscle paralysis, and sometimes are difficult to distinguish from one another. Misdiagnosis leads to mismanagement as the three diseases need different specific treatments and approaches to the prevention of recurrent attacks. HypoPP includes familial HypoPP and sporadic HypoPP. Familial HypoPP is an autosomal dominant hereditary disorder. It may result from one of the following mutations: in the skeletal muscle α1 subunit of the dihydropyridine receptor calcium channel gene (*CACNL1A3*), including DII/S4 Arg528His, DIV/S4 Arg1239His and DIV/S4 Arg1239Gly [1–3]; in the α subunit of the tetrodotoxin-sensitive voltage-dependent sodium channel gene (*SCN4A*), including DII/S4 Arg669His, DII/S4 Arg672His and DII/S4 Arg672Gly [4,5]; or in the potassium channel, Arg83His [6]. Most of the cases of HypoPP in Western countries are familial forms, but most Asian HypoPP is sporadic [7]. The cause of sporadic HypoPP is still unknown. It is unclear if sporadic HypoPP is a subgroup of familial HypoPP whose penetrance is incomplete.

TPP is another type of hypokalaemic periodic paralysis. It is associated with clinically overt

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hyperthyroidism [8] and is also more prevalent in Asians [8–10]. Hypokalaemia in HypoPP and TPP is caused by intracellular potassium shifts. The last syndrome, dRTA, is characterized by persistent hyperchloraemic metabolic acidosis and urinary potassium loss. The syndrome results from impaired acid excretion in the absence of renal insufficiency [11]. The syndrome occurs sporadically, and environmental factors may play an important role [12].

We therefore performed a prospective study of patients presenting in our institute with hypokalaemia and severe paralysis in order to determine the causes, clinical characteristics and common mutations in our HypoPP and TPP patients.

Subjects and methods

Subjects

All patients who presented in the Ramathibodi Hospital, Bangkok, between 1997 and 2002 with severe limb paralysis and serum potassium <3 mmol/l were enrolled in the study. Severe limb paralysis was defined as an acute loss of muscle strength severe enough to prevent standing without assistance. The exclusion criterion was a serum creatinine >1.2 mg/dl.

Methods

Clinical presentations and patients' characteristics were documented during attacks of paralysis and hypokalaemia, and blood samples were obtained from the patients to test for electrolytes, blood urea nitrogen, creatinine, calcium, phosphate, magnesium and thyroid function, including T4, FT4, T3 and TSH. In addition, 24 h urine collections were studied for volume, sodium, potassium and creatinine. A long acid loading test was performed in all patients who were euthyroid after hypokalaemia was corrected. The long acid loading tests were performed by administering 0.1 g/kg/day of NH_4Cl orally for 3 days to induce systemic acidosis, as previously described by Wrong and Davies [13]. The diagnosis of dRTA was based on an abnormal long acid loading test, which is defined as the failure to acidify urine (pH >5.5) and a urinary excretion of ammonia <50 mEq/day in the presence of systemic acidosis (blood pH <7.35). Incomplete dRTA patients were defined as patients who had abnormal long acid loading tests and who did not have acidosis (serum bicarbonate >18 mmol/l) at the time of presentation [14].

Mutation study

We screened 15 unrelated HypoPP and 11 TPP patients for common mutations; 50 healthy Thai blood donors served as normal controls.

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The primers Ca11F and Ca11R (5′-CAA CCC TAC AGA CAT TGC CAA C-3′ and 5′-GGC ACT CAC TTG GTG ATC TTG A-3′), and Ca30F and Ca30R (5′-GT TCT GCC CCC AGA CTG AC-3′ + 5′-GGA CTT GAT GAA CGT CCA CA-3′) were used to amplify portions of the DIIS4 and DIVS4 segments which contain, respectively, *Bbv*I and *Nla*III restriction sites. PCR products were digested and analysed as in a previous report [15].

Single strand conformation analysis (SSCA) and sequencing. Exons 11 and 30 of *CACNL1A3*, exon 12 of *SCN4A* and exon 1 of *KCNE3* were screened for SSCA. The primer sequences for amplifying exon 12 of *SCN4A* were 5′-CCA GGA GGT GGG AGT TGG GT-3′ and 5′-TGG CCC TGG GCT TTT GTG TA-3′. For exon 1 of *KCNE3*, two previously reported overlapping PCR fragments were used [6]. Each PCR fragment was screened by using a thermoflow electrophoresis temperature control system (Novex, San Diego, CA), and it was separated on a 20% polyacrylamide gel and visualized by silver staining. The SSCA variants were then sequenced directly using an ABI 3100 DNA sequencer.

PCR sequencing. To screen the R1239H/R1239G mutations in *CACNL1A3* and R669H, R672H/R672G in *SCN4A*, exons 30 of *CACNL1A3* and 12 of *SCN4A* were amplified. For R83H in *KCNE3*, the second half of exon 1 was amplified. PCR products were sequenced directly.

Polymorphism analysis. For silent polymorphisms at nucleotides 1551 and 1564, the variants were confirmed as in a previous report [15]. Polymorphism 1491 did not alter a restriction site. The primer combinations Ca11F and Ca11RM (5′-ACC ACG AAG CAG TCG AAT CG-3′) were used to amplify for ARM assay [16]. This variant created a new restriction site for the restriction enzyme *Bsp*D1.

The study protocol was approved by the Ramathibodi ethics conmmittee. Written informed consent was obtained from all patients.

Statistical analysis

All data are expressed as mean ± SD. Differences in group means were compared using one-way analysis of variance (ANOVA) with Bonferroni's correction. Differences in categorical variables were compared using Fisher's exact test. The difference was considered significant if the *P*-value was <0.05.

Results

There were 47 patients recruited for the study; nine patients did not complete the tests. Four patients were diagnosed as having Bartter's syndrome, primary hyperaldosteronism, current usage of a diuretic, and gastrointestinal potassium loss, respectively. Consequently, of the original selection, 34 patients completed the tests and were followed-up. Thyroid function and the long acid loading tests indicated that 15, 11 and eight were HypoPP, TPP and dRTA, respectively. All patients were diagnosed for the first time during this study. Both HypoPP and TPP patients had 24 h urine potassium of <20 mmol/day during the attack, indicating that there was no urinary loss of potassium.

Table 1. Clinical features of patients in the HypoPP, TPP and dRTA groups

	HypoPP	TPP	dRTA
Numbers	15	11	8
Male:female	13:2	10:1	1:3
Northeastern origin	11 (73%)	3 (27%)	5 (62%)
Positive family history	5 (33%)	1 (9%)	0
Age at first attack (years)			
11–20	6	1	0
21–40	8	6	5
>40	1	4	3
Mean age at first attack (years)	22.4 ± 8.0	31.9 ± 12.4	40.8 ± 11.7
No. of attacks			
1	5 (33%)	3 (27%)	4 (50%)
2–10	6 (40%)	7 (63%)	2 (25%)
>10	4 (26%)	1 (9%)	2 (25%)
Precipitating causes of first attack			
Exercise/heavy work	8	3	2
High carbohydrate intake	4	3	2
Emotional stress	2	5	0
Alcohol intake	1	0	1
Unknown	0	0	3
Time of first attack			
Day (6 a.m.–6 p.m.)	3 (20%)	6 (54%)	3 (37.5%)
Night (6 p.m.–midnight)	3 (20%)	1 (9%)	2 (25%)
During sleep	9 (60%)	4 (36%)	3 (37.5%)
Recovery time after treatment			
<24 h	14	9	2
1–3 days	1	2	6
Previous history of hyperthyroidism	0	2 (18%)	0
Exopthalmos	0	2 (18%)	0
Goitre	0	1 (9%)	0
Kidney stone/nephrocalcinosis	0	0	3 (37%)
Serum potassium at presentation (mmol/l)	2.39 ± 0.46	2.61 ± 0.68	2.36 ± 0.37

HypoPP = hypokalaemic periodic paralysis, TPP = thyrotoxic periodic paralysis; dRTA = distal renal tubular acidosis.

Table 1 summarizes the clinical characteristics of each group of patients. There was a significant difference in the ages at the first attacks between HypoPP and dRTA ($P = 0.001$, ANOVA). There were also significant differences in the male to female ratios among the three groups ($P = 0.003$). Eleven of the 15 patients with HypoPP (73%) and five out of eight patients with dRTA (62%) were originally from northeastern Thailand. In contrast, only three out of 11 patients (27%) in the TPP group were of northeastern origin. One-third of the HypoPP patients had positive family histories of paralysis, while this was the case for only one TPP patient. None of the dRTA patients had a positive family history. Most HypoPP (14 out of 15) and TPP patients (nine out of 11) recovered from hypokalaemia and severe paralysis, being able to walk within 24 h after treatment. However, the recovery time in dRTA patients was 1–3 days after treatment.

In the TPP group, only two out of 11 patients (18%) had previous histories of hyperthyroidism. Of those 11 patients, seven (63%) had clinically overt hyperthyroidism at presentation, while the other four (37%) had subtle clinical hyperthyroidism despite biochemical confirmation of thyrotoxicosis. Positive thyroid antimicrosomal or anti-thyroglobulin antibodies, or both, were detected in seven patients (64%).

Table 2 demonstrates blood chemistries and long acid loading test results in dRTA and HypoPP patients. In the dRTA group, two patients had spontaneous systemic acidosis, and six did not show metabolic acidosis at presentation (serum bicarbonate <18 mmol/l), but had abnormal long acid loading tests, being consistent with incomplete dRTA. None of the dRTA patients was positive for anti-Ro and anti-La.

Mutation analysis

No common mutations were detected by PCR-RFLP, SSCA and PCR sequencing. However, aberrant bands were noted by SSCA in exon 11 of *CACNL1A3* in both HypoPP and TPP patients (Figure 1A). Direct sequencing of the PCR products showed three silent polymorphisms including C1491T, C1551T and C1564T located at nucleotides 92, 32 and 19 upstream of R528H (Figure 1B). The polymorphisms C1491T and C1551T are present in both homozygotes and heterozygotes, but the C1564T polymorphism is only present in heterozygotes. The frequencies of the variants are shown in Table 3. Two of the three silent polymorphisms (C1551T and C1564T) found in our population were similar to those reported in Brazil [15]. However, the third one (C1491T) was unique

Table 2. Patients' blood chemistries and long acid loading test results in the dRTA and HypoPP groups

	Age[a] (years)	Electrolyte at presentation (mmol/l)				Baseline $U.NH_4Cl$ (mmol/day)	After long acid loading test			
		Na	K	Cl	CO_2		$U.NH_4Cl$ (mmol/day)	U.pH	B.pH	$S.HCO_3^-$ (mmol/l)
dRTA group										
Mean ± SD	40.8 ± 11.7	140.1 ± 3.9	2.36 ± 0.37	110.1 ± 6.3	19.4 ± 3.3	32.6 ± 6.0	40.0 ± 7.7	5.91 ± 0.37	7.29 ± 0.07	12.6 ± 3.4
HypoPP group										
Mean ± SD	22.4 ± 8.0	141.1 ± 4.5	2.39 ± 0.46	107.7 ± 4.5	21.0 ± 2.5	45.1 ± 11.9	96.3 ± 21.6	5.10 ± 0.16	7.30 ± 0.04	13.1 ± 1.6

[a]Determined at the time of the first attack of paralysis.
$U.NH_4Cl$ = urine ammonium chloride; $S.HCO_3^-$ = serum bicarbonate concentration; U.pH = urine pH; B.pH = blood pH; dRTA = distal renal tubular acidosis; HypoPP = hypokalaemic periodic paralysis.

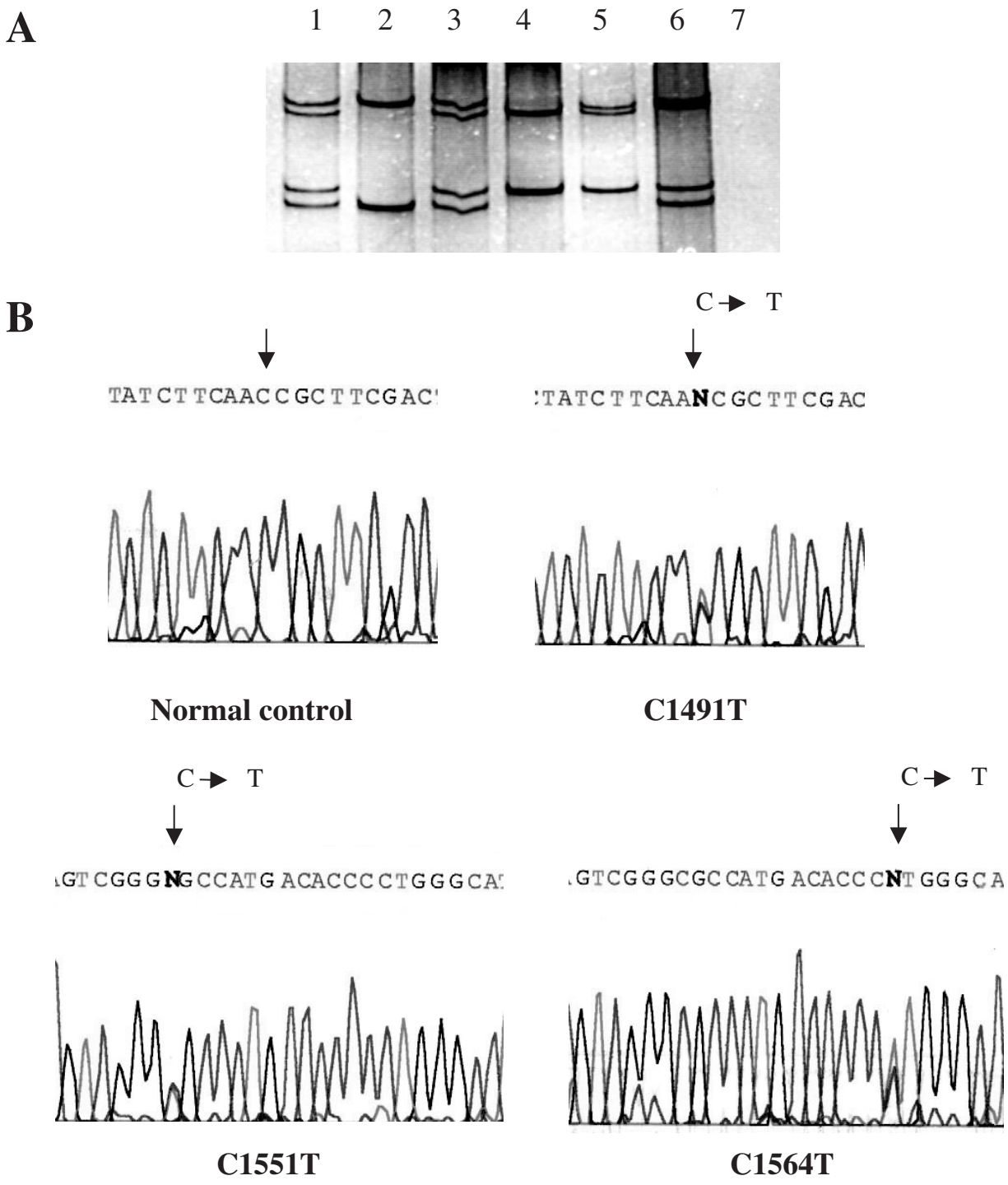

Fig. 1. Polymorphisms in *CACNL1A3*. (**A**) SSCA patterns of the polymorphisms. Lane 1, wild-type; lane 2, C1491T homozygous; lane 3, C1491T heterozygous + C1551T heterozygous; lane 4, C1551T homozygous; lane 5, C1551T heterozygous + C1564T heterozygous; lane 6, C1491T heterozygous + C1564T heterozygous; lane 7, water. (**B**) DNA sequence analysis of each polymorphism. Vertical arrows indicate the position of change.

in our population. The frequencies of C1551T and C1564T in our controls were quite similar to those reported previously, 18 and 4% in Thailand versus 18 and 8.6% in Brazil, respectively. We did not find an over-occurrence of C1551T in our HypoPP patients compared with the controls. However, we found that the frequencies of C1551T were higher in our TPP patients compared with the controls, 45 versus 18% ($P < 0.05$). The latter result was similar to data reported previously [15].

Table 3. Polymorphisms in *CACN1A3* found in HypoPP and TPP in the Thai population

Nucleotide	Normal control ($n=50$)	HypoPP ($n=15$)	*P*-value	TPP ($n=11$)	*P*-value
C1491T	8 (16%)	3 (20%)	0.71	3 (27%)	0.37
C1551T	9 (18%)	2 (13%)	0.67	5 (45%)	<0.05
C1564T	2 (4%)	0	0.43	1 (9%)	0.49

HypoPP = hypokalaemic periodic paralysis; TPP = thyrotoxic periodic paralysis.

Discussion

HypoPP can be considered a type of skeletal muscle 'channelopathy'. Several mutations have been reported in the skeletal muscle dihydropyridine receptor calcium channel (*CACNL1A3*) [1–3], the tetrodotoxin-sensitive voltage-dependent sodium channel (*SCN4A*) [4,5] and the potassium channel (*KCNE3*) [6]. However, sporadic cases without documented evidence of mutations have also been reported [6,17]. In Taiwan, most cases of HypoPP are sporadic [7]. The sporadic cases are more frequent in men than women. This is in agreement with our findings since only one-third of our HypoPP group showed a positive family history, and most of our HypoPP patients were males. Most of the HypoPP patients reported from the West had familial forms, whereas those reported from Asia were predominantly sporadic. In both our familial and sporadic HypoPP patients, we failed to identify common mutations that have been reported in the literature. This raises the possibility that HypoPP in different ethnic settings may be linked to different types of mutations or to different environmental factors. Among patients in the West, an abnormal sodium or potassium channel itself may cause abnormal ion transport and account for the paralysis, as demonstrated previously for the *SCN4A* and *KCNE3* gene mutations [4,6]. Although hypokalaemia in our HypoPP patients was probably caused by a cellular shift of potassium, since all patients had 24 h urine potassium <20 mmol/day, the hypokalaemia might be caused by as yet unknown environmental factors. It is clear that more genetic studies are required before a definite conclusion can be drawn.

The clinical manifestations of TPP are similar to those of HypoPP, and they subside after the euthyroid state is restored. It is reasonable to suspect that TPP might be associated with molecular defects in calcium, sodium or potassium channels, as described for familial HypoPP; however, common mutations were not identified in our TPP patients. These data are concordant with the results reported previously by Dias da Silva *et al.* [15], who failed to identify common mutations in the calcium channel in TPP patients.

In the case of dRTA, most patients in our population were sporadic and idiopathic. Genetic factors are less likely to be involved, as only a minority of dRTA patients can be linked by genetic analysis [18]. Environmental factors may be the predominant causes [12].

The mean ages in our HypoPP, TPP and dRTA patients are in agreement with data from previous reports [7,10,19]. The HypoPP group had the lowest mean age at first attack. The male gender was six times more common than the female gender in the HypoPP patients, and 10 times more common in those with TPP, whereas females were three times more common than males in the cases of dRTA. The male predominance in HypoPP and TPP agrees with results reported previously [7,8–10].

Based on the above results, if the patient is female, the diagnosis of dRTA is more likely. Conversely, if the patient is male, age at onset of the first attack and his geographic residence will provide clues to the diagnosis. However, a thyroid function test and a long acid loading test would be required for definite diagnosis.

The dRTA group required a longer time to recover after treatment of their hypokalaemia than did the HypoPP and TPP groups. This phenomenon may be explained by the fact that dRTA patients lost significant amounts of body potassium, while HypoPP and TPP patients did not have much change in their total body potassium.

In our study, the majority of patients in the TPP group had no previous history of thyroid disease, and some had only subtle clinical hyperthyroidism. These findings are consistent with what was reported previously from Hong Kong [10]. In the dRTA group, five out of eight patients showed neither metabolic acidosis nor stone/nephrocalcinosis despite abnormal urinary acidification. This could imply that more than half of dRTA patients who presented with hypokalaemia and paralysis may have been misdiagnosed. This finding indicates that the acid loading test should be a part of the work-up of the syndrome of hypokalaemia and paralysis.

In conclusion, our study demonstrated that, in Thailand, a syndrome of hypokalaemia and severe paralysis may result from HypoPP, TPP or dRTA. Most HypoPP cases are sporadic and are not associated with any common mutations reported previously. HypoPP in different ethnic backgrounds may be asssociated with different types of mutations or may result from environmental factors. There are some clinical clues to differentiate between HypoPP, TPP and dRTA, including age at onset, gender, geographic residence and associated findings. However, clinical features alone cannot definitely differentiate one from the others. The absence of a history of thyroid disease or clinical thyrotoxicosis does not exclude the diagnosis of TPP. In addition, the absence of stone/nephrocalcinosis or metabolic acidosis does not exclude the diagnosis of dRTA. It is emphasized that definite diagnosis depends on an awareness both of the causes of the syndrome and of the necessary elements of a thorough work-up.

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Conflict of interest statement. None declared.

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