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SLC34A3 Intronic Deletion in an Iranian Kindred with Hereditary Hypophosphatemic Rickets with Hypercalciuria and Review of Reported Cases

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What is already known on this topic?
Hereditary Hypophosphatemic Rickets with Hypercalciuria (HHRH) is a very rare inheritable hypophosphatemic rickets/osteomalacia. Biallelic mutations in SLC34A3/NPT2c gene are responsible for the occurrence of the disease.
What this study adds?
In this paper, we describe the clinical examination, biochemical profile and gene analysis of Iranian kindred with a 101bp deletion in SLC34A3 gene. Genetic counseling and screening for SLC34A3 mutations can be helpful in adult onset phenotype with unexplained osteoporosis, bone deformities and especial recurrent renal stones.

Abstract
Objective: Hereditary Hypophosphatemic Rickets with Hypercalciuria (HHRH) is a very rare inheritable hypophosphatemic rickets/osteomalacia characterized by decreased renal phosphate reabsorption, hypophosphatemia, vitamin D refractory rickets, hyperphosphaturia, hypercalciuria, elevated circulating 1, 25-dihydroxy vitamin D levels and low serum parathyroid hormone (PTH) levels, leading to growth retardation, limb deformities, bone pain, muscle weakness, rickets and osteomalacia. Biallelic mutations in SLC34A3/NPT2c gene are responsible for the occurrence of the disease.
Methods: In this paper we describe the clinical examination, biochemical profile and gene analysis of Iranian kindred with a 101bp deletion in SLC34A3 gene. 12 members of a family of previously reported patient with HHRH (3 homozygote and 7 heterozygote) and 10 healthy controls were evaluated.
Results: All patients had significantly increased risk of kidney stone formation, bone deformities and short stature compared with unrelated healthy controls. The heterozygous patients displayed milder clinical symptoms compared with homozygous patients. These patients displayed mild or no hypophosphatemia and they did not develop skeletal deformities. Recurrent renal stones and hypercalciuria were the main presentations of heterozygous patients which could be confused with familial hypercalciuria. In addition, biochemical analysis showed significantly low serum sodium level and elevated alkaline phosphatase in patients.

Conclusion: Genetic counseling and screening for SLC34A3 mutations can be helpful in adult onset phenotype with unexplained osteoporosis, bone deformities and especial recurrent renal stones. In subjects with vitamin D deficiency the results should be interpreted cautiously.
Key words: Hereditary hypophosphatemic rickets with hypercalciuria, SLC34A3 gene, Hypophosphatemia, Hypercalciuria.

Introduction

Loss of function in the third member of the sodium phosphate cotransporter family type II, NaPi-IIc/NPT2c, which is encoded by the SLC34A3 gene causes Hereditary hypophosphatemic rickets with hypercalciuria (HHRH) (1). HHRH is a rare metabolic disorder (OMIM #241530) with an autosomal recessive mode of inheritance that was first described in large consanguineous Bedouin kindred (1-3). The candidate gene which is located on chromosome 9q34 expresses at the apical domain of renal proximal tubule cells and plays fundamental roles in the maintenance of phosphate homeostasis (2, 4). NPT2c contributes to renal phosphate reabsorption from glomerular filtrate under the hormonal control of parathyroid hormone (PTH) and the fibroblast growth factor 23 (FGF23) (2). Phosphate participates in a remarkably wide array of cellular processes, intracellular signaling, pH buffering, bone mineralization, phospholipids structures, and nucleic acids synthesis (4). Clinically, HHRH patients, who carry homozygous or compound heterozygous SLC34A3/NPT2c mutations, often show hypophosphatemia following decreased renal phosphate reabsorption, rickets and/or osteomalacia, frequently kidney stones or nephrocalcinosis. Hypophosphatemia is followed by upregulation of renal 1-α-hydroxylase and increased serum level of 1,25-dihydroxy vitamin D (1,25(OH)2 D) results in elevated intestinal absorption of calcium and urinary calcium excretion despite suppressed parathyroid function (1, 5, 6). High serum 1,25(OH)2 D concentrations and hypercalciuria distinguish HHRH from other hypophosphatemic disorders (7). Other features include slow growth, limb deformities, muscle weakness, bone pain, bowing, and short stature (5, 8, 9). From the initial description few families and sporadic cases have been reported in Turkey, Holland, Morocco, North America, Japan, Africa, Caucasus, Germany and Iran (2, 9-11). This study reports a case series in a kindred describing clinical features, biochemical profile and subsequent candidate gene analysis of a family with a 101-bp intronic deletion within the SLC34A3 gene.

Methods

Patients and data collection

12 members of a family of previously reported patient (11) with HHRH (Figure 1) were evaluated in the endocrine unit of the Shariati Hospital, Tehran University of Medical Sciences. Analysis of the extended family discloses other members with a history of nephrolithiasis. Ten unrelated healthy subjects were included in this study as control group (No deformity, renal stone and history of calcium and bone disease). The study was approved by the Ethical Committee of Endocrinology and Metabolism Research Institute, Tehran University of Medical Sciences. Written informed consent was obtained from the family.

Clinical assessment

A detailed clinical examination was conducted to identify the presence of any physical signs and symptoms of rickets including; skeletal deformities, leg pain, difficulty walking, bow-leg. Height and weight of patients was measured. Skeletal X- rays were taken and renal ultrasonography and radiological examination were performed. Bone mineral density (BMD) of spine, right hip and forearm was assessed for two homozygous patients using dual-energy X-ray absorptiometry (DEXA) method.

Biochemical assay

The biochemical laboratory evaluation was performed. 12-hour overnight fast blood and 24h urine samples were collected to measure calcium, creatinine and phosphorus. Concentration of intact PTH, serum 25-hydroxyvitamin D [25(OH)D], total alkaline phosphatase were assessed. Fasting tubular reabsorption of phosphate (TRP), maximal renal phosphate reabsorption per glomerular filtration rate (TMP/GFR), was calculated using the following formula: 1 - (urine phosphorus × serum creatinine/serum phosphorus × urine creatinine).

Genetic analysis

Genomic DNA was isolated from peripheral blood leukocytes. 101bp deletion in intron 9 of the SLC34A3 gene was screened after direct sequencing of the entire SLC34A3 gene on ABI 3130 genetic analyzer (8).

Statistical analysis

Normal distribution of continuous variables was assessed using Kolmogorov–Smirnov test. Continuous variables with normal distribution are presented as mean (SD). Comparison of continuous variables between groups was done using ANOVA test. All statistical analysis was performed using SPSS software version 16. P-value less than 0.05 considered as statistically significant.

Results

A total of 12 individuals, 3 homozygote, 7 heterozygote and 2 healthy members of an HHRH kindred were included in this study. Genetic analysis revealed presence of 101-bp deletion in intron 9 (Figure 1). Bone mineral densitometry (BMD) of spine, right hip and forearm in 2 homozygous patients was measured (Table 1) and very low bone mineral density and osteoporosis was found. The T score of spine, right hip and forearm was -1.6 and -3.4, -2.7 and -2.3, -3.9 and -3.7 in patients 3-3 and 3-2, respectively. Table 2 showed clinical examination and biochemical tests carried out on the mutant homozygous and heterozygous individuals of investigated kindred. Seven members of kindred had history of kidney stone. Serum creatinine was high in two members (3-1 and 3-3) and serum calcium was high in other two members (4-1 and 4-3). High alkaline phosphatase was seen in most of kindred members. Comparison of biochemical examinations between mutant homozygous and heterozygous individuals in the kindred and healthy controls was shown in Table 3. Serum concentrations of sodium and alkaline phosphatase and MCHC were significantly different among three groups ($P<0.05$). Serum concentrations of sodium, potassium and calcium were lower in homozygous individuals compared to normal individuals. Serum alkaline phosphatase was higher in homozygous and heterozygous individuals in comparison with healthy controls ($P=0.008$). Whereas increased hematocrits and low serum phosphate levels were not significant. 24 hrs urine volume and urine calcium were higher in homozygous patients.

Homozygote and heterozygote mutations in the SLC34A3 gene lead to a significantly increased risk of kidney stone formation and bone deformities leading to short stature and growth delay, compared with ten unrelated healthy controls.

Discussion

In this case series study, we describe the clinical examination, biochemical profile and gene analysis of the family members (affected and unaffected) of an HHRH patient to establish etiology. A total of 12 individuals in Iranian kindred were included in this study, 3 homozygote, 7 heterozygote and 2 healthy members.

Homozygote and heterozygote mutations in the SLC34A3 gene lead to a significantly increased risk of kidney stone formation and bone deformities, compared with healthy controls. A hallmark feature of familial hypophosphatemic rickets is short stature resulting from deformity and growth retardation, which is observed in homozygous and heterozygous individuals. Hypophosphatemia in HHRH leading to elevation in the serum level of 1,25(OH)2D which resulted in hypercalciuria. Episodes of hypercalciuria may cause in development of recurrent renal stones. Based on biochemical follow-up data that were available for the homozygote and heterozygote members in this kindred, significantly low serum sodium levels and elevated alkaline phosphatase was observed.

The causative gene, SLC34A3, which is mapped on chromosome 9q34 encodes a member of SLC34A transporter family of proteins which is involved in transporting phosphate into cells mediated by sodium-phosphate cotransporters in the renal brush border membrane, and plays a key role in phosphate homeostasis, despite its low expression levels (4, 8). It has been demonstrated that homozygous for the disrupted NPT2 gene mice show many of the features of HHRH and concluded that the slc34A1/Napi7 gene plays a key role in phosphate homeostasis and in normal skeletal development (12). Not many cases of Biallelic SLC34A3/NPT2c mutations, HHRH syndrome, have been reported worldwide (5). Different mutations in SLC34A3/NPT2c with different phenotypes have been reported in these patients (1, 2, 5, 6, 8-10, 13-17). This study reports the first kindred of HHRH in Iran and describes a reported mutation, a 101bp deletion, within the SLC34A3 gene, which affects transcription or splicing of pre-mRNA, causes aberrant RNA splicing, between exons 9 and 10.

Since HHRH is an autosomal recessive disease, biallelic mutations are required for full-scale disease manifestations; loss of one SLC34A3 allele does not always lead to laboratory abnormalities. However, clinical phenotypes are sometimes seen in carriers of single SLC34A3 mutations (2, 6). Similarly, several heterozygous members of the Bedouin kindred for the c.228delC mutation, displayed mild hypophosphatemia, reduced TmP/GFR, and elevations in 1,25(OH)2D levels in addition to increased urinary calcium excretion (8). In the present study, the heterozygous patients displayed milder clinical symptoms compared with homozygous patients. These patients displayed mild or no hypophosphatemia and they did not develop skeletal deformities. Recurrent renal stones and hypercalciuria were the main presentations of these patients which could be confused with familial hypercalciuria. HHRH diagnosis can be missed in this situation and appropriate interpretation of the clinical symptoms is so important. Absence of clinical symptoms and of biochemical alterations have also been reported in previous heterozygous HHRH families (2, 5, 6, 8, 17). Therefore, genetic tests screening for SLC34A3 mutations can be helpful in patients with suspicious clinical findings.

Hypophosphatemia and renal stones are common in homozygous patients. However, bone deformities may not develop in all of these patients. It can make it difficult to diagnose this disease. More clinical examinations and genetic evaluation are needed in this situation too.

Vitamin D level and/or rising in serum creatinin could be two critical key points in serum and urine levels of phosphorous and calcium. As reported in patients 3-1 and 3-3 chronic kidney diseases and vitamin D deficiency are two important issues for interpretation of biochemical findings. Undesirable vitamin D status is highly prevalent among Iranian adults which vitamin D deficiency and insufficiency was reported in 90.7% of the adult population (18). Moreover, hypercalciuria and renal stones are prevalent in Iran from childhood (19, 20). Therefore, these issues could be effective on the biochemical presentation of this disease.

Large number of studied individuals is strength of this study. However, sample size of the control cohort is small. One of the limitations of the present study was lack of clinical and genetic testing for all members of the family. Moreover, the concentration of 1,25(OH)2D3 which is very important in differentiating HHRH patients, was not measured in this study. It should be noted that the 24 hr. urine samples are influenced by dietary phosphate and 3 hr. fasting spot urines were not determined in order to calculate %TRP. In addition, genes encoding other phosphate transporters were not sequenced.

In conclusion, as the clinical phenotype of HHRH can be quite variable with different penetrance even in the same family with identical mutations, not possible to be certain about a genotype–phenotype effect, proper diagnosis required molecular genetic analysis. Screening for SLC34A3 mutations to know the importance of treatment and close follow-up to avoid complications can be helpful.

Conflict of interests: There is no conflict of interest to declare.

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Table 1. Comparison of bone mineral densitometry (BMD) values (g/cm^2) of spine, right hip and forearm in 2 homozygous patients

			Patient 3-3 (age:33)	Patient 3-2 (age:30)
Spine	Total	BMD	0.910	0.672
		T-Score	-1.6	-3.4
		Z- Score	-1.6	-3.4
Right Hip	Neck	BMD	0.238	0.538
		T-Score	-5.1	-2.8
		Z- Score	-4.8	-2.7
	Troch	BMD	0.264	0.525
		T-Score	-4.1	-1.8
		Z- Score	-3.9	-1.8
	Total	BMD	0.629	0.657
		T-Score	-2.7	-2.3
		Z- Score	-2.6	-2.3
Forearm	Total	BMD	0.485	0. 378
		T-Score	-3.9	-3.7
		Z- Score	-3.8	-3.6

Table 2. Clinical examination and biochemical findings of the mutant homozygous and heterozygous individuals

	Reference range(Adult)	2-1 (Homo)	2-✸ (Hetero)	2-2 (Hetero)	2-3 (Hetero)	3-1 (Hetero)	3-2 (Homo)	3-3 (Homo)	3-4	3-5	4-1 (Hetero)	4-2 (Hetero)	4-3 (Hetero)
age		50	60	56	53	26	30	33	36	38	3	9	13
sex		Female	Male	Female	Female	Male	Female	Male	Female	male	Female	Female	Female
Height		147	173	150	153	178	135	150					158
Weight		68	63	55	72	95	47	55		75		22	40
Kidney stone		No	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	No	No	No
deformity		Yes	No	No		No	Yes	Yes	No	Yes		No	No
Serum creatinine(mg/dl)	0.3-1.40	0.87	0.89	1.00	0.83	1.97	0.78	2.26		1.20	0.53	0.63	0.73
Serum calcium(mg/dl)	8.9-10.1 (1-14 years: 9.5-10.6)	9.40	8.90	9.50	9.30	9.80	8.60	9.60		9.70	10.60	9.70	10.60
Serum phosphorus(mg/dl)	2.7-4.5 (1-12 years: 3-6)	3.00	3.20	3.10	3.00	3.90	5.10	3.00		2.30	4.70	4.40	4.80
Alkaline phosphatase(u/liter)	Female:64-306 Male:80-306 (1-15 years: 180-1200)	537.00	255.00	359.00	245.00	319.00	357.00	407.00		578.00	512.00	715.00	267.00
Parathyroid hormone(pg/ml)	10-62	18.00	29.00	30.00	196.00	100.00	36.00	66.00		29.00	37.00	38.00	35.00
Serum 25-hydroxy vitamin D(ng/ml)	20-100	62.00	99.10	118.00	12.00	25.00	12.80	19.30		22.00	59.00	19.00	16.00
Urine creatinine(mg/ 24h)	600-1800	611.00	1071.00	585.00	933.00	1817.00	693.00	966.00		1272.00		376.00	864.00
Urine calcium(mg/ 24h)	Up to 300 (Up to 4 mg/kg)	188.00	289.00	221.00	50.00	48.00	209.00	172.00		29.70		56.00	92.00
Urine phosphorus(mg/ 24h)	-	266.00	799.00	312.00	518.00	667.00	385.00	907.00		468.00		252.00	328.00
FEp		0.10	0.20	0.17	0.15	0.18	0.08	0.70		0.19		0. 09	0.05

TRP	0.85-0.98	0.89	0.79	0.82	0.84	0.81	0.91	0.29		0.81		0.90	0.92
GFR		97.70	77.00	64.14	104.00	76.35	92.00	36.2		86		63.50	95.89
TMP/GFR (mg/ dl)	2.5-4.2	3	2.7	2.7	2.3	3.2	5	0.9		1.8		4.4	4.8
Three subjects (4-1, 4-2, 4-3) are younger than 13 years old. Interpretation for biochemical tests should be considered in the reference range of this group. 2-1, 3-2 and 3-3 are homozygote and 2-*, 2-2, 2-3, 3-1, 4-1, 4-2 and 4-3 are heterozygote.													

Table 3. Comparison of biochemical findings between mutant homozygote and heterozygote and normal controls

Variables	**Normal**	**Homo**	**Hetero**	**P-value**
Weight	61.33(8.13)	56.67(10.59)	57.83(25.37)	0.88
WBC	5.78 (1.01)	6.53(2.49)	7.21(1.86)	0.24
RBC	5.04(0.42)	5.62(0.51)	5.21(0.53)	0.22
Hemoglobin	14.53(1.42)	16.07(2.00)	14.54(1.89)	0.38
Hematocrit	42.93 (3.18)	48.67(5.41)	45.01(4.79)	0.14
MCV	85.56(8.11)	86.5(2.47)	86.3(4.04)	0.96
MCH	28.95(3.05)	28.53(1.00)	28.87(2.27)	0.71
MCHC	33.79(0.88)	33.00(0.52)	32.26(1.49)	0.04
Platelets	263.88(41.25)	259.00(38.74)	276.43(110.24)	0.92
FBS	100.44(5.93)	79.33(3.51)	109.28(60.77)	0.52
Urea	32.33(7.48)	30.00(13.07)	34.14(11.04)	0.82
Creatinine	0.95(0.14)	1.30(0.83)	0.94(0.48)	0.43
Triglyceride	110.55(58.10)	142.00(87.11)	113.14(55.85)	0.74
Total Cholesterol	208.66(55.48)	227.00(38.97)	206.28(43.99)	0.82
HDL Cholesterol	56.88(8.79)	56.33(7.23)	57.00(7.44)	0.99
LDL Cholesterol	117(36.73)	123.33(24.4)	111.57(34.01)	0.88
Sodium	142.11(1.96)	138.43(0.58)	138.57(1.45)	0.001
Potassium	4.28(0.32)	3.87(0.47)	4.17(0.11)	0.12
Calcium	9.8(0.39)	9.20(0.53)	9.77(0.64)	0.22
FEp	0.15(0.05)	0.29(0.2)	0.14(0.04)	0.24
TRP	0.84(0.05)	0.69(0.19)	0.85(0.17)	0.45
GFR	89.85 (30.24)	75.3(0.16)	80.15(21.5)	0.35
Alkaline Phosphatase	198.0(63.87)	433.66(92.91)	381.71(173.40)	0.008
Phosphorus	3.82(0.43)	3.70(1.21)	3.87(0.77)	0.94
PTH	58.7(44.81)	40.0(24.2)	66.4(62.3)	0.75
25-OH VitD	39.0(20.8)	31.4(26.7)	49.7(43.4)	0.67
Urine Volume(24 hrs)	1333(582.5)	2200(1735)	1250(677)	0.28
Urine Creatinine(24hrs)	990(328)	757(186)	941(497)	0.66
Urine Calcium(24 hrs)	152(110)	189(18.5)	126(103)	0.67
Urine Phosphorus(24 h)	657.7(313)	506(356)	479(220)	0.48

Variables are presented as mean (SD).
Three heterozygous individuals are younger than 13 years old and they are not included in the analysis.
WBC, White blood cells; RBC, Red blood cells; MCV, Mean corpuscular volume; MCH, Mean corpuscular hemoglobin; MCHC, Mean corpuscular hemoglobin concentration; FBS, Fasting blood sugar; FEp, Free erythrocyte

protoporphyrin; TRP, Tubular reabsorption of phosphate; GFR, Glomerular filtration rate; PTH, parathyroid hormone.

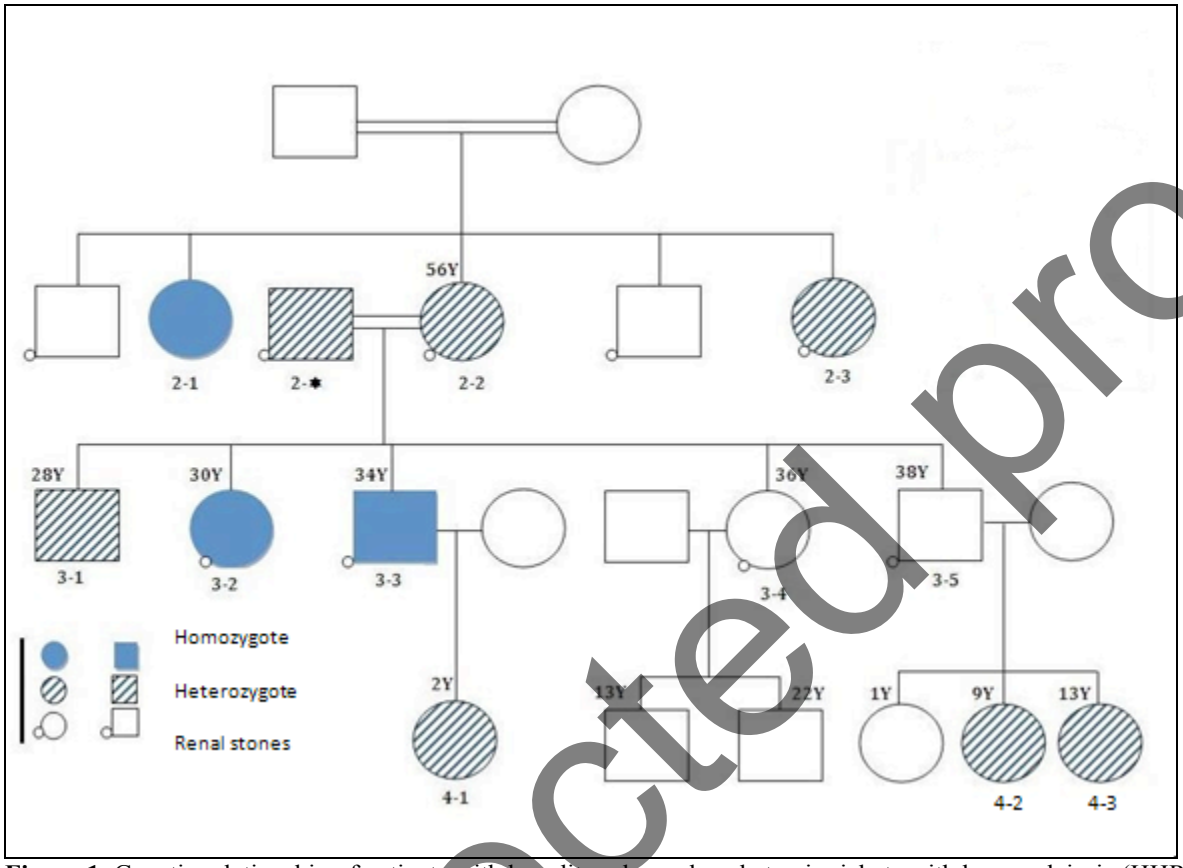

Figure 1. Genetic relationship of patients with hereditary hypophosphatemic rickets with hypercalciuria (HHRH). Genetic analysis was not done for subject 3-5.