Familial Hypocalciuric Hypercalcemia: The Challenge of Diagnosis

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Abstract

Familial hypocalciuric hypercalcemia (FHH) is an autosomal dominant genetic disorder classically characterized by lifelong mild-to-moderate asymptomatic hypercalcemia with inappropriately normal to elevated serum parathyroid hormone (PTH) concentrations and hypocalciuria, best expressed by a urine calcium-to-creatinine clearance ratio (CCCR)<0.01[1,2]. FHH prevalence is estimated between 1:10 000 to 1:100 000[3,4]. In 60% of cases, FHH is due to *CASR* inactivating mutation[5]. More rarely FHH is due to *AP2S1* or *GNA11* inactivating mutation, both genes encoding for proteins involved downstream of *CASR* activation[6]. These molecular alterations are found in all parathyroid cells, explaining disease persistence following partial parathyroidectomy and the ineffective surgical management of these patients. FHH phenotypes could however overlap with primary hyperparathyroidism (PHPT). Indeed, even if patients with FHH are currently asymptomatic, some of them present chondrocalcinosis, kidney stones or bone fracture and very high level of PTH or calcemia[7]. Nonetheless, the distinction has to be adressed since the therapeutic approach significantly differs between these two conditions. Surgery is usually recommended for PHPT[8] while follow-up is preferred in the latter case[9,10]. We report and discuss 7 cases, 6 out 7 being operated for a presumed PHPT.

Introduction

Familial hypocalciuric hypercalcemia (FHH) is an autosomal dominant genetic disorder classically characterized by lifelong mild-to-moderate asymptomatic hypercalcemia with inappropriately normal to elevated serum parathyroid hormone (PTH) concentrations and hypocalciuria, best expressed by a urine calcium-to-creatinine clearance ratio (CCCR) < 0.01[1, 2]. FHH prevalence is estimated between 1:10 000 to 1:100 000[3, 4]. In 60% of cases, FHH is due to *CASR* inactivating mutation[5]. More rarely FHH is due to *AP2S1* or *GNA11* inactivating mutation, both genes encoding for proteins involved downstream of *CASR* activation[6]. These molecular alterations are found in all parathyroid cells, explaining disease persistence following partial parathyroidectomy and the ineffective surgical management of these patients. FHH phenotypes could however overlap with primary hyperparathyroidism (PHPT). Indeed, even if patients with FHH are currently asymptomatic, some of them present chondrocalcinosis, kidney stones or bone fracture and very high level of PTH or calcemia[7]. Nonetheless, the distinction has to be adressed since the therapeutic approach significantly differs between these two conditions. Surgery is usually recommended for PHPT[8] while follow-up is preferred in the latter case[9, 10]. We report and discuss 7 cases, 6 out 7 being operated for a presumed PHPT.

Methods

We performed a retrospective analysis of patients with hyperparathyroidism managed in the department of Endocrine Surgery that were found to carry *CASR* variants between 2016–2020. Our surgery department is a high volume endocrine surgery deparment with approximately 300 parathyroid interventions per year. The study was approved by the Assistance Publique-Hôpitaux de Marseille ethics advisory committee (PADS21-46).

Results

Seven index cases (5F/2M, mean age 53.4y) with *CaSR* variants were identified, 6 of which had undergone surgical intervention. Six out of 7 could be classified as FHH with identification of new pathogenic or likely pathogenic variants, while the 7th case had a parathyroid adenoma (PA) with a *CASR* variant that was ultimately classified as variant of uncertain significance (VUS). All had hypercalcemia with inappropriately normal or high PTH 1-84 values (Table 1).

Three patients presented with a classical FHH phenotype. In the first case (1:F-45y), the disease occurred in a context of IgA nephropathy with progressive decline of kidney function. Surgical intervention was indicated after a 5 years follow-up in our nephrology tertiary referral center, where hypocalciuria was misinterpreted as being secondary to chronic kidney disease, in the presence of a single parathyroid abnormality visible on both neck ultrasound (US) and parathyroid scintigraphy. Calcemia was 2.64mmol/L(N:2.20-2.55mmol/L) with high PTH level(68.8pg/ml,N:15-65pg/ml). Minimally invasive surgery was converted to open surgical intervention due to insufficient decrease of intraoperative PTH, and a subtotal parathyroidectomy (PTx) (3 glands removed) was performed.

The second patient (2:M-76y) had a previous history of resection of a parathyroid adenoma. His latest follow-up showed a persistent hypercalcemia (2.65mmol/L) with hypophosphatemia (0.75mmol/L,N:0.81-1.45mmol/L). Calciuria was in the normal range limit (103 and 160mg/day,N:100-300mg/day) and his CCCR (0.017) was inconclusive to differentiate between FHH and PHPT. The patient had renal lithiasis, osteopenia and chronic renal failure stade 2. Neck US and parathyroid scintigraphy were negative. Because of the presumed persistent PHPT with negative parathyroid imaging, a genetic screening was performed. Genetic testing for case 2 revealed the same pathogenic *CASR* variant as case 1(c.893C>T,p.(Ala298Val))[7], a finding which argued against reoperation.

The third patient (3:M-77y) had a classical FHH biochemical phenotype (calcemia=2.61mmol/L,PTH=70pg/mL,calciuria=72mg/day,CCCR=0.007). Imaging were negative. Genetic testing revealed a *CASR* pathogenic variant in exon 5(c.1525G>A,p.(Gly509Arg))[11].

Three additional cases had undergone surgical intervention due to a less typical clinical picture with marked hypercalcemia and fluctuating values of calciuria. All of them were relatively young at diagnosis and had negative parathyroid imaging studies despite significant hypercalcemia (2.80,2.90,2.84mmol/L,respectively). Bilateral neck exploration was performed which resulted in resection of two hyperplastic glands in cases 4 and 6, and one hyperplastic gland in case 5. All had persistent hypercalcemia post-operatively. It has to be underlined that the existence of a multiglandular disease,

objectivated during cervicotomy, prompted the surgeons to limit the extent of parathyroid resection to prevent the occurrence of post-operative hypoparathyroidism. Genetic counseling enabled the identification of relatives with *CASR* variants.

In case 4 (F-42y), post-operative calciuria (400mg/24h) and CCCR (0.021) were not decreased. Genetic testing detected a pathogenic *CASR* variant(c.1664T>C,p.(Ile555Thr))[7,12]. Her daughter, 20y, had a mild hypercalcemia (2.63mmol/L), hypophosphatemia (0.65mmol/L), mild elevated PTH (73pg/mL), normal calciuria (144mg/24h) with a decreased CCCR (0.008). Her son, 18y, had significant hypercalcemia (2.88mmol/L), normal PTH (58pg/mL), normal calciuria (171mg/24h) and a decreased CCCR (0.006). Again, both children had negative parathyroid scintigraphy.

In another patient (5:F-36y) a *CASR* variant(c.511A>T,p.(Ser171Cys)) was identified. It was present in her mother, her sister, her brother and her niece. followed-up in our institution and has a marked hypercalcemia (2.94mmol/L).

The 6th patient (6:F,52y) had a *CASR* variant(c.1664T>C,p.(Ile555Thr)). Her brother carried the same variant and had hypercalcemia (2.80mmol/L) with hypocalciuria (68mg/day).

Taken together, these cases (4-6) illustrate that the presence of hypercalcemia in relatives during family screening is very powerful indicator suggesting for the diagnosis of FHH. Negative parathyroid imaging together with marked hypercalcemia and calciuria in the low normal range could also raise the suspicion of FHH.

Finally, the last case (7: F-46y) had typical features of PHPT: calcemia>3mmol/L with a marked elevation of serum PTH 1-84 (629pg/ml), hypophosphatemia (0.74mmol/L) and a normocalciuria (234mg /day) with a high CCCR (0.026). Work-up revealed an osteopenia, a stage 3 renal insufficiency with normal kidney ultrasound. Neck US and parathyroid scintigraphy were concordant for a left P4-derived adenoma. A large left P4 adenoma, i.e adenoma on left superior parathyroid gland (weight:580mg), was resected via minimally-invasive surgery with a significant decline in intraoperative PTH 1-84. Genetic testing revealed an unknown *CaSR* variant in exon 3(c.347C>T,p.(Ala116Val)) classified as VUS. Phosphocalcic imbalance was normalized following surgery. Although, we could consider in the present case that *CASR* may be not involved in the patient's pathology, physicians should be aware that this rare variant may be not always pathogenic.

Discussion

This series illustrates the heterogeneity in FHH biochemical phenotypes with a possible overlap with PHPT that could lead to inappropriate management. Beyond biochemical characterization, parathyroid imaging also plays an important role in parathyroid disease subtyping. In PHPT, a negative parathyroid imaging study should raise the suspicion of multiglangular hyperplasia[13,14]. This series shows that negative parathyroid imaging is part of the clinical picture of FHH. In France, FHH genetic testing is recommended in all patients presenting with familial PHPT or isolated PHPT before 50 years old. This involves the simultaneous exploration of genes involved syndromic hyperparathyroidism (i.e.*MEN1*, *CDKN1B*, *CDC73*)[15]. Genetic testing should be also performed in case of suspicion of FHH or in doubtful clinical situations or in patients with persistent hypercalcemia following PTx. However, despite technological improvements in genetic sequencing, it does not appear reasonable to submit all hyperparathyroid patients to genetic analysis. In our insitution, we are in agreement with the guidelines that recommend performing genetic testing for patients with one of the following criteria[16]:

- Age<50y
- Family history of hypercalcemia
- Hyperparathyroidism with CCCR<0.01 and/or calciuria below 1.33mg/day/kg using 24-hour urine collection

However, based on this case series, we would like to emphasize that age at diagnosis has a limited diagnostic value, presence of single gland abnormality on parathyroid imaging is not synonym of PHPT and presence of *CASR* variant is not synonym of FHH.

Addtionally, we consider that :

1. FHH screening is unnecessary if simultaneous calciuria and CCCR are above 4mg/kg/24h and 0.02, respectively. Indeed, looking specifically at CCCR and 24h-calciuria, we found very few patients [7,17] with a real FHH (i.e.without single parathyroid adenoma) and simultaneous CCCR>0.02 and 24h-calciuria>0.1mmol/Kg
2. In the presence of serum total calcium ≥2.8mmol/L, a negative parathyroid imaging should raise the suspicion of FHH.
3. In case of doubt regarding the disease, assessement of calcemia in first degree relatives is of powerful value and may guide genetic testing.
4. HPT patients, especially this negative or discordant imaging findings, should be discussed at a multidisciplinary team meeting in order to ensure proper management.

Declarations

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Declaration of interest

The authors have declared no conflicts of interest.

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Tables

Table 1 – Clinical, biological, imaging and genetic characteristics of FHH patients

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7
Sex	F	M	M	F	F	F	F
Age (years)	45	76	77	42	36	52	46
Previous familial history of hypercalcemia	No	No	No	No	No	No	No
Relative cases	No	No	No	Yes	Yes	Yes	No
Comorbidities	IgA nephropathy	T2D	T2D, HTA	No	No	Hypertension	HTA
Medications	ACE inhibitor	Metformin, IDPP4	Sulfuronylure-Calcic inhibitor ARA2	No	No	ARA2 Thiazidic	B-blockers
Weight (kg)	50	75	82	54	54	63	82
Calcium pre-operative (mmol/L)	2.64	2.69	2.61	2.8	2.90	2.68	3.08
Calcium post-operative	2.50	2.65	-	2.75	2.83	2.78	2.25
Phosphore pre-operative(mmol/L)	0.88	NA	0.67	0.55	0.57	0.73	1.0
Phosphore post-operative	1.34	0.75	-	0.77	0.80	1.0	1,23
vitamin D (nmol/L)	119	39	60	45	80	97	42
PTH pre-operative(pg/mL)	68.8	44.3	70	98	42.4	68.8	629
PTH post operative (pg/mL)	98	97	-	93,1	71	69	47
PTH per-operative (pg/mL)	70.7 ->24.5	NA	-	216->67.9	96.2 -> 43.4	67.9->44.3	327.2-> 46.2
Creatinine (mcmol/L)	116	102	73	56.1	57	76	114
eGFR (mL/min)	49	66	96	112	116	78	50
Calciuria (mg/day)	19	103	72	183	106	187	234
Calciuria (mg/kg/day)	0.38	1.37	0.87	3.32	1.96	2.96	2.85
CCCR	0.004	0.017	0.007	NA	NA	NA	0,026
Osteopenia/osteoporosis	No	Yes	Yes	Yes	NA	Yes	Yes
Femoral T-score	0.4	-1.1	0.4	0.1	NA	NA	-1.1
L1-L4 T-score	1.2	0	-1.4	-1.5	NA	NA	-0.4
Renal lithiasis	No	Yes	No	No	Yes	No	No
Neck ultrasound	Left P3	Negative	Negative	Negative	Negative	Negative	Left P4
Parathyroid scintigraphy	Left P3	Negative	Negative	Negative	Negative	Negative	Left P4
Histopathology	Hyperplasia	Adenoma	-	Hyperplasia	Hyperplasia	Hyperplasia	Adenoma
Uniglandular/Multiglandular disease	Multiglandular	Uniglandular	-	Multiglandular	Uniglandular	Multiglandular	Uniglandular
CASR Mutation - Exon	CaSR exon 4	CaSR exon 4	CaSR exon 5	CaSR exon 6	CaSR exon 4	CaSR exon 6	CaSR exon 3
Nucleotide variant	c.893C>T	c.893C>T	c.1525G>A	c.1664T>C	c.511A>T	c.1664T>C	c.347C>T
Protein variant	p.(Ala298Val)	p.(Ala298Val)	p.(Gly509Arg)	p.(Ile555Thr)	p.(Ser171Cys)	p.(Ile555Thr)	p.(Ala116Val)
Status	heterozygous	heterozygous	heterozygous	heterozygous	heterozygous	heterozygous	heterozygous
Classification	Probably pathogenic	Probably pathogenic	Pathogenic	Pathogenic	Probably Pathogenic	Pathogenic	Unknown

T2D : Diabete type 2 , HTA : Hypertension

eGFR : estimated glomerular filtration rate, calculated according to the CKD-EPI formula.
CCCR : Calcium creatinine clearance ratio.

Previous family history of hypercalcemia was assessed by interwiewing index case and relative-cases were determined by performing phosphocalcic blood test and genetic research after detection of the mutation on the index case.
Femoral T-score is referring to femoral neck T-score.

P3 is defined by inferior parathyroid gland, P4 is defined by superior parathyroid gland

Normal range of biological value : Calcemia (2.20-2.55 mmol/L) – Phosphore (0.81-1.45mmol/L) – PTH (15-65 pg/ml) – Vitamin D (75-250 nmol/L) – Calciuria (100-300mg/day)

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