Clinical characteristics of familial hypocalciuric hypercalcaemia type 1: A multicentre study of 77 adult patients

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Abstract

Objective: Familial hypocalciuric hypercalcaemia type 1 (FHH1), related to heterozygous loss-of-function mutations of the calcium-sensing receptor gene, is the main differential diagnosis for primary hyperparathyroidism. The aim of our study was to describe clinical characteristics of adult patients living in France with a genetically confirmed FHH1.

Design and patients: This observational, retrospective, multicentre study included 77 adults, followed up in 32 clinical departments in France, with a genetic FHH1 diagnosis between 2001 and 2012.

Results: Hypercalcaemia was diagnosed at a median age of 53 years [IQR: 38-61]. The diagnosis was made after clinical manifestations, routine analysis or familial screening in 56, 34 and 10% of cases, respectively, (n = 58; data not available for 19 patients). Chondrocalcinosis was present in 11/51 patients (22%), bone fractures in 8/56 (14%) and renal colic in 6/55 (11%). The median serum calcium was 2.74 mmol/L [IQR: 2.63-2.86 mmol/L], the median plasma parathyroid hormone level was 4.9 pmol/L [3.1-7.1], and the median 24-hour urinary calcium excretion was 2.8 mmol/24 hours [IQR: 1.9-4.0]. Osteoporosis (dual X-ray absorptiometry) or kidney stones (renal ultrasonography) were found in 6/38 patients (16%) and 9/32 patients (28%), respectively. Fourteen patients (18%) underwent parathyroid surgery; parathyroid adenoma was found in three patients (21%) and parathyroid hyperplasia in nine patients (64%). No correlation between genotype and phenotype was established.

Conclusion: This large cohort study demonstrates that FHH1 clinical characteristics can be atypical in 33 patients (43%). Clinicians should be aware of this rare differential diagnosis in order to adopt an appropriate treatment strategy.

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KEYWORDS
calcium-sensing receptor, familial hypocalciuric hypercalcaemia, parathyroid adenoma, primary hyperparathyroidism

1 | INTRODUCTION

In a patient given a biochemical diagnosis of primary hyperparathyroidism (PHPT), familial hypocalciuric hypercalcaemia (FHH), albeit rare, should always be considered in the differential diagnosis. FHH, inherited as an autosomal dominant trait, was first described by Foley in 1972[1] and is characterized by a slightly elevated serum calcium, with inappropriate plasma parathyroid hormone (PTH) mostly in the reference range, very low 24-hour urinary calcium excretion and the absence of clinical morbidities such as osteoporosis or kidney stones. In 65% of patients, FHH is due to heterozygous inactivating mutation in the gene encoding the calcium-sensing receptor (CaSR), leading to type 1 FHH (FHH1, OMIM#145980).[2] Although parathyroidectomy is considered as a first-line treatment in PHPT, surgery is ineffective in FHH. Therefore, the correct diagnosis is important in order to avoid ineffective or even deleterious surgical management strategies. The calcium/creatinine clearance ratio (CCCR) has been put forward to differentiate the two entities, but an overlap is observed, especially for values between 0.01 and 0.02.[3] Thus, a risk equation has recently been developed in an attempt to distinguish between these two entities.[4]

Atypical characteristics including chondrocalcinosis, kidney stones or very high levels of PTH or serum calcium[5,6] were described in some patients. These overlaps could complicate differential diagnosis between these two conditions in the clinical setting. Moreover, parathyroid adenomas have been reported in patients with *CASR* inactivating mutation (Table 1).

The aim of this study was to describe the clinical characteristics in a large cohort of French adult patients with genetically confirmed FHH1.

2 | PATIENTS AND METHODS

2.1 | Design

Between 2001 and 2012, all patients over 18 years of age with *CASR* inactivating mutation diagnosed in 3 French centres performing *CASR* genotyping were considered for inclusion in this observational, retrospective, multicentre study.

This study was conducted in accordance with the Declaration of Helsinki and approved by the local Ethics Committees of Toulouse University Hospital (authorization number 24-0413, granted on 6 May 2014) and by the Commission Nationale de l'Informatique et des Libertés (French Data Protection Authority) (CNIL DR-2014-343). In accordance with French legislation, all patients gave their informed consent to undergo genetic testing.

2.2 | Patients

One hundred and sixty-four records of patients aged 18 years or more at the time of the genetic diagnosis of *CASR* inactivating mutation were included in this study. The patients' files were retrospectively reviewed by one investigator (CM). Eighty-four patients were excluded: 45 patients because of absence of response from their medical centres and 39 patients due to insufficient data in patients' files. Data were collected for 80 patients (48% of the initial population), followed up in 32 French Endocrinology, Nephrology or Rheumatology departments. Finally, three patients were excluded due to uncertain significance of the genetic variant that was found.

2.3 | Methods

The following data were recorded: circumstances under which hypercalcaemia was diagnosed, clinical symptoms, evaluation of renal and/or bone characteristics, laboratory results, parathyroid imaging and neck surgery results.

Biochemistry: The following data were collected: total and ionized calcium, albumin, protein, creatinine, phosphate, PTH, calcidiol, calcitriol, magnesium plasma levels and 24-hour urinary calcium, creatinine and phosphate excretion measured by standard laboratory methods used in each centre. We adjusted total serum calcium for individual variations in serum albumin or in proteins. The albumin-adjusted calcium concentration was calculated as adjusted calcaemia (mmol/L) = total calcaemia (mmol/L) – 0.025 × (plasma albumin (g/L) – 40). The adjusted calcium protein level was calculated as follows: adjusted calcaemia (mmol/L) = total calcaemia (mmol/L)/(0.55 × (plasma protid (g/L)/160). Creatinine clearance was calculated using the MDRD (Modification of Diet in Renal Disease) formula. The calcium to creatinine clearance ratio was calculated by multiplying the urine calcium/creatinine ratio by the serum creatinine/calcium ratio. For each item of data, we selected the results, which were the closest to the time of diagnosis of the hypercalcaemia.

Dual-energy X-ray absorptiometry (DXA): bone mass density T score and Z score (measured with equipment from each centre) were

TABLE 1 Patients with FHH1 presenting a histologically confirmed parathyroid adenoma, reported in literature and in our study

	1	2	3	4	5	6	7
Sex	F	M	F	F	F	M	F
Age at hypercalcaemia	63	61	76	75	20	76	45
Genotype	R220P	R25X	E250K	E250K	Q926R	p.(Ser1065 Valfs*11)	C562Y
Reference	Egan et al, 2013[22]	Frank-Raue et al, 2011[21]	Frank-Raue et al, 2011[21]	Frank-Raue et al, 2011[21]	Frank-Raue et al, 2011[21]	Yabuta et al, 2009[23]	Burski et al 2002[24]
Symptomatology or Comorbidities at diagnosis	• Nephro-lithiasis (at 33 years) • Osteopaenia • Chronic constipation	• Nephro-lithiasis • HBP	• Nephro-lithiasis • HBP • Osteoporosis (vertebral fractures)	= 0	HBP	• Nephro-lithiasis	• Constipation • Fatigue • PUPDS • Psychiatric disorder (personality trouble, depression) • Gastric ulcer • HBP
Results before parathyroid surgery							
Ca (mmol/L)	3.25	2.90	2.90	3.0	3.3	2.80	4.48
PTH (pmol/L)	15	9	17	43	21	14	63
Ca U (mmol/24h)	4.8	NA	NA	NA	NA	NA	NA
CCCR	NA	0.031	0.031	0.025	0.027	0.005	0.008
Cervical morphologic examinations	MIBI +	US and/or MIBI +	US and/or MIBI +	US and/or MIBI +	US and/or MIBI +	US, MIBI, cervical scanner +	US–MIBI +
Age at parathyroid surgery	71	NA	NA	NA	23	76	NA
Results after parathyroid surgery							
Follow-up of symptoms after parathyroid surgery	Fatigue and psychiatric troubles improved	NA	NA	NA	NA	NA	NA
Ca (mmol/L)	2.79	2.90	2.30	2.20	2.30	2.68	2.72
PTH after surgery (pg/mL)	68	40	40	63	41	54	73
Ca U (mmol/24h)	2.2	1.8	0.3	NA	NA	NA	NA
CCCR	0.008	0.004	0.009	NA	0.015	0.004	0.002

Note: Normal reference ranges in present study: Total serum calcium: 2.2-2.6 mmol/L; PTH: 1.1-6.8 pmol/L; 24-h urinary calcium excretion: 2.5-7 mmol/24 h

Abbreviations: Ca U: 24-hour urinary calcium excretion; Ca: total serum calcium; CCCR: calcium creatinine clearance ratio (urinary calcium/plasma calcium x plasma creatinine/urinary creatinine); F: female; HBP: high blood pressure; M: male; PUPDS: polyuro-polydipsic syndrome; US: ultrasonography.

measured at three sites: lumbar (L2-L4 or L1-L4), femoral and radial; if these data were not available, osteoporosis or bone demineralzsation reported in the medical record was taken into account. Osteoporosis was defined by a T score below −2.5 SD, and osteopaenia by a T score between −1 and −2.5 SD, in men and women over 50 years of age. In men and women under 50 years of age, bone demineralization was defined by a *Z* score below −2 SD.

Genetics: Genomic DNA was isolated from white blood cells using standard procedures. Coding exons 2-7 and the immediate flanking intron sequence were amplified and sequenced bidirectionally as previously described.[4,7,8] Sequences were aligned to the GenBank CASR reference sequence NM_000388.3. Variants not described in the HGMD database http://www.hgmd.cf.ac.uk/ac/gene.php?gene=CASR were classified using the American College of Medical Genetics (ACMG) Guidelines.[9] The ACMG recommendations to classify sequence variants is the method used worldwide to interpret variants in genes responsible for Mendelian disorders in the clinical setting. ACMG recommends the classification of

8	9	10	11	12	13	14	15	16
M	F	M	F	M	F	F	M	F
16	NA	57	56	35	56	35	72	15
E297K	E297K	C395R	P748R	I32V	I283T	G557E	R680C	T640I
Brachet et al, 2009[25]	Brachet et al, 2009[25]	Forde et al, 2014[26]	Eldeiry et al, 2012[27]	Eldeiry et al, 2012[27]	Guarnieri et al, 2010[18]	Present study	Present study	Present study
• Fatigue • Abdominal pain • PUPDS		• Fatigue • Joint pain • Insomnia	NA	• Fatigue • Decreased tolerance exercise • Osteopaenia	• HBP • Nephro-lithiasis (at 36 years) • Osteoporosis without fracture	• Digestive troubles • Bone demineralization	• Fatigue • Digestive troubles • HBP • Cognitive troubles	• Fatigue • Digestive troubles
3.50	3.25	2.61	2.87	2.97	2.98	3.14	3.3	3.01
27	NA	9	7	9	15	13	11	3
NA	NA	NA	2.3	5.2		4.1	0.2	4.8
0.009	NA	NA	0.0047	0.0096	0.025	NA	0.0011	0.01
US− MIBI +	NA	US + MIBI +	US + MIBI +	US + MIBI + (possible)	US +	US + MIBI +	US + MIBI +	MIBI−
16	55	57	56	45	56	36	73	25
NA	NA	Unchanged	NA	NA	PUPDS and psychiatric troubles improved	NA	Cognitive troubles improved	
2.70	2.68	2.87	2.65	2.67	2.45	2.81	2.56	2.60
69	74	59	42	47	NA	81	64	32
NA	NA	2.3	2.5	1.8	2.5	NA	NA	NA
0.001	0.004	<0.01	0.0045	0.0038	NA	NA	NA	NA

variants into five categories (class 1: benign; class 2: likely benign; class 3: uncertain significance; class 4: likely pathogenic and class 5: pathogenic) based on a combination of several criteria including population data, computational data, functional data and segregation data. Class 4 and 5 variants were required to confirm FHH diagnosis, using the website: http://www.medschool.umaryland.edu/Genetic_Variant_Interpretation_Tool1.html/. All the variants described in this cohort were carefully revised with the criteria of ACMG classification.

Statistics: Results were reported as medians and interquartile ranges (IQR). Qualitative values were compared using Fisher's exact test; quantitative values were compared between 2 groups using the Mann-Whitney test for unmatched data and Wilcoxon's test for matched data; quantitative values were compared between 3 groups using the Kruskal-Wallis test. Dependence between quantitative variables was analysed with Spearman's rho correlation coefficient (r). Statistical analyses were carried out using GraphPad version 5 software for Windows (La Jolla

California USA). The *P*-value was two-sided and the cut-off value of significance was .05.

3 | RESULTS

3.1 | Clinical characteristics

Seventy-seven patients were enrolled including 49 females (64%). The median age at diagnosis of hypercalcaemia was 53 years (IQR: 38-61; range: 15-76 years). The main diagnostic circumstances are summarized in Figure 1. Hypercalcaemia was detected during a routine check-up or fatigue investigation in 20/58 patients (34%; data not available for 19 patients) (Figure 1). A familial history of hypercalcaemia, PHPT or neck surgery was documented in 32/56 patients (57%) on diagnosis, with one family member presenting FHH in 6/56 cases (11%) (Figure 2).

3.2 | Kidney, bone and other comorbidities

Six patients had a history of renal colic. Their 24-hour urinary calcium excretion (median 3 mmol/24 h; IQR: 2.36-3.37) did not differ significantly from those recorded in patients without history of renal colic (median 2.80 mmol/24 h; IQR: 1.79-4.30; *P* = 1.00). Kidney stones were found in 9 out of 32 patients (28%) who had a renal ultrasonography; they were bilateral in 5 out of nine patients; no nephrocalcinosis was detected; three of these patients had been symptomatic with an episode of renal colic.

Osteoporosis was diagnosed in six female patients; 3 of them were between 62 and 72 years of age at the time of diagnosis (no data available for the other three patients). In 38 patients who underwent DXA (median age: 58 years; 12 males and 26 females; 10 patients below 50 years of age), the median *Z* score was 0.20 SD (IQR: −0.85 - 1.06) in the lumbar spine (n = 20), −0.15 SD (IQR: −0.43 - 0.53) in the femoral neck (n = 20) and −0.85 SD (IQR: −1.48 - −0.11) at the one-third distal radius site (n = 8). Bone characteristics at DXA showed an osteoporosis in 6/35 patients (17%), an osteopaenia (T score between −1 and − 2.5 SD at age >50 years) in 11/35 (31%) and a low bone mineral density at age <50 years (*Z* score < −2SD) in 2/35 (6%). Eight out of 56 patients sustained a fracture (14%); 2 of these patients were suspected to have been osteoporotic (vertebral and femoral neck) before the age of 55. There were no significant clinical or laboratory differences between osteoporotic patients and nonosteoporotic patients, except for the age at which hypercalcaemia was diagnosed (63 years; IQR: 56-65 for osteoporotic patients, vs. 45 years; IQR: 39-56 for non-osteoporotic patients; *P* = .039).

Other comorbidities were chondrocalcinosis (n = 11/51; 22%), psychiatric disease (n = 18/55; 33%) and hypertension (n = 29/57; 51%). The prevalence of these comorbidities in our study is higher than the prevalence observed in the general population.

3.3 | Laboratory characteristics

A median maximum serum calcium of 2.88 mmol/L (IQR: 2.78-3.00) and a median minimum serum calcium of 2.59 mmol/L (IQR: 2.52-2.71) were recorded (normal range: 2.20-2.60). There was a significant correlation between the minimum and maximum serum calcium for the same patient (rho: 0.52; p: 0.0001). The serum total calcium level was above 2.9 mmol/L in 11/77 patients (14%) (Table 2).

Plasma PTH was above 10.6 pmol/L (100 pg/mL) in 6/66 patients (9%) (normal range: 1.1-6.8).

Twenty-four-hour urinary calcium excretion was elevated (>0.1 mmol/kg/24 h) in 4/42 patients (10%) (Table 2). The CCCR was below 0.01 in 15/30 patients (50%) and above 0.02 in 3/30 patients (10%).

There was no correlation between laboratory characteristics and gender or age, except for a weak correlation between age and PTH (rho = 0.33; *P* = .015). Serum calcium did not differ statistically between patients with or without comorbidities.

3.4 | Morphological characteristics and therapeutic management

Neck ultrasonography and MIBI scintigraphy were performed in 42 and 39 patients, respectively. Neck ultrasonography led to the localization of one (or more) enlarged parathyroid gland(s) in 24% of patients (n = 10/42), and MIBI scintigraphy led to the detection of one (or more) pathological hyperfixation in 26% of patients (n = 10/39).

Thirteen out of 77 patients (17%) received a medical treatment with cinacalcet. With a median dose of 45 mg/day [IQR: 30-60], it led to a median serum calcium reduction of 0.44 mmol/L [IQR: 0.31-0.55] after 2 to 6 months treatment and to a normalization of serum calcium in 7 out of nine patients with available data.

Surgery was performed in 14/77 patients (18%): 1 to 3.5 parathyroid glands per patient were removed. Twenty-nine glands were analysed histologically: 10 were normal (34%), 16 were hyperplastic (55%), and 3 were adenomatous (10%). Surgery was performed prior to genetic diagnosis in every patient except one; genetic diagnosis was obtained within a median period of 12 months [IQR: 3-40] after surgery. Surgery had been performed because of increased serum calcium above 2.80 mmol/L in 11 out of 14 patients with available data (and >3 mmol/L in 4 of them). Moreover, two patients presented with bone comorbidities (osteoporosis and/or osteoporotic fractures), one patient presented with cognitive disorders, two patients presented with renal comorbidities (kidney stones and/or history of renal colic), two patients presented with chondrocalcinosis and one patient had undergone one episode of acute pancreatitis at the age of 24 years (Table 3). Morphological examinations did not predict the presence of a histologically abnormal parathyroid gland. One of the morphological examinations performed found one abnormal parathyroid gland in the two patients with four histologically normal parathyroid glands. On the contrary, the morphological

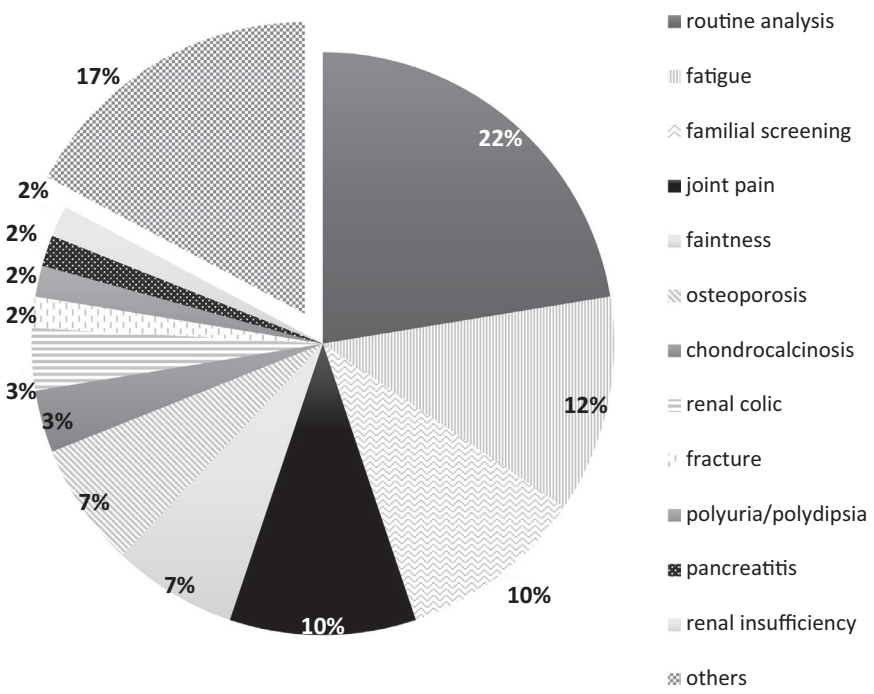

FIGURE 1 Circumstances leading to diagnosis of hypercalcaemia (data available for 58 patients)

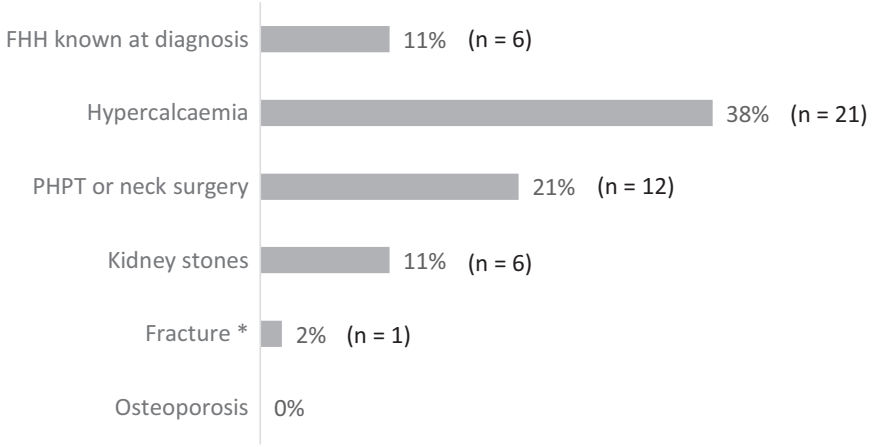

FIGURE 2 Family history (data available for 56 patients). (* Fracture considered: fracture whose site suggests osteoporosis). FHH: familial hypocalciuric hypercalcaemia; PHPT: Primary hyperparathyroidism

examinations did not detect any abnormal parathyroid gland in 5/11 (45%) patients with at least one histologically abnormal parathyroid gland. Serum calcium returned to the normal range in two patients after surgery; a parathyroid adenoma was histologically confirmed in these two patients, and the serum calcium was still normal at the last visit, 2 and 10 years after surgery, respectively (Table 1). In five patients, one hyperplastic gland was found (after removal of 1, 2 or 3.5 parathyroid glands). In three patients, 2 or more hyperplastic glands were found (after removal of 2, 3 or 3.5 parathyroid glands). Calcium levels did not return to the normal range in any of these patients.

3.5 | Typical and atypical characteristics of FHH

In this study, 51/77 patients (66%) presented with one or more typical criteria of FHH such as a familial history of hypercalcaemia or parathyroid surgery, and/or a 24-hour urinary calcium excretion below 3.5 mmol/24 h. However, 33/77 patients (43%) presented with an atypical FHH characteristic such as hypercalciuria >7 mmol/24 h or >0.1 mmol/kg/24 h, CCCR >0.02, serum calcium >3 mmol/L, a high PTH level > 10.6 pmol/L (100 pg/mL), presence of kidney stones or osteoporotic bone fracture(s), evidence of parathyroid adenoma on neck ultrasonography or MIBI scintigraphy, or a surgically excised parathyroid adenoma.

3.6 | Genetic characteristics

The median age at the time of the genetic diagnosis was 57 years (IQR: 42-65). Sixty different mutations were identified in this series; 50/60 (83%) were missense mutations (Table 4). They were mostly localized in the extracellular domain (35/60; 58%). Ten (17%) of these mutations were identified in 2 or more patients (regardless of family connections), without any phenotype-genotype correlation

TABLE 2 Laboratory characteristics

	Median (IQR)	Available Data number	Considered reference range	N (%) <lower normal limit	N (%) >upper normal limit
Plasma					
Total serum calcium (mmol/L)	2.74 [2.63-2.86]	77	2.2-2.6	0 (0%)	58 (76%)
Albumin-adjusted calcium (mmol/L)	2.72 [2.64-2.86]	36	2.2-2.6	0 (0%)	30 (83%)
Ionized calcium (mmol/L)	1.45 [1.35-1.52]	26	1.18-1.34	0 (0%)	20 (77%)
Phosphate (mmol/L)	0.88 [0.78-1.05]	56	0.8-1.5	19 (34%)	1 (2%)
Creatinine clearance (mL/min/1.73 m^2)	93 [70-103]	39	60-120	6 (15%)	4 (10%)
Magnesium (mmol/L)	0.91 [0.84-0.97]	23	0.70-1.05	1 (4%)	1 (4%)
Calcidiol (nmol/L)	40 [30-63]	45	50-250	37 (82%)	0 (0%)
Calcitriol (pmol/L)	108 [80-158]	25	45-150	1 (4%)	8 (32%)
PTH (pmol/L)	4.9 [3.1-7.1]	66	1.1-6.8	0 (0%)	18 (27%)
Urine					
Creatinine (mmol/24 h)	7.1 [5.5-11.1]	31			
Calcium (mmol/24 h)	2.8 [1.9-4.0]	55	2.5-7	25 (45%)	2 (4%)
Calcium (mmol/kg/24 h)	0.046 [0.026-0.055]	42	< 0.1		4 (10%)
Calcium/creatinine (mmol/mmol)	0.34 [0.17-0.45]	30	< 0.6		3 (10%)
CCCR	0.009 [0.006-0.011]	30	0.01-0.02	15 (50%)	3 (10%)
Phosphate (mmol/24 h)	21.6 [17.2-31.3]	31	16-32	6 (19%)	8 (26%)

Note: For each variable, the first result during the first 6 mo of follow-up was collected.

Abbreviations: CCCR: calcium creatinine clearance ratio (urinary calcium/plasma calcium x plasma creatinine/urinary creatinine); PTH: parathyroid hormone.

being identified. Actually, 16/60 (16%) *CASR* variants had not been yet described and identified in terms of function and 12 of them are missense. All of them were class 4 or class 5 variants.

4 | DISCUSSION

A large cohort of patients with genetically proven FHH1 is described in this paper. In this study, 51/77 patients (66%) presented with one or more typical FHH criteria. However, 33/77 patients (43%) had atypical FHH characteristics.

First, in our study, hypercalcaemia is not generally diagnosed at a young age. Indeed, the median age for diagnosis is 53 years; only 10% of patients were diagnosed through familial screening, and the age at which hypercalcaemia is diagnosed varies according to diagnostic circumstances. Marx *et al* found that hypercalcaemia was diagnosed at 48 ± 6.1 years of age among index cases,[10] which is similar to the age observed in our cohort, compared to 37 ± 7 years in Simonds' cohort.[5]

Secondly, we show that kidney and bone comorbidities are not rare in FHH. In a Danish series of 100 patients with familial hypocalciuric hypercalcaemia (data available for 66 patients), osteoporosis was diagnosed in 6 to 9% of cases[3]; this prevalence is lower than that recorded in our study, but DXA was performed at a younger age in these patients. In our cohort, the Z score at lumbar and femoral sites indicated a bone mineral density similar to that of the general population at the same age. Another French FHH1 series highlighted a history of kidney stones in 4/52 patients.[7] In our study, more patients presented with kidney stones on renal ultrasonography (28%). Clinical or radiological chondrocalcinosis was found to be highly prevalent in our study, which suggests a higher risk of joint impairment. To our knowledge, this has not yet been reported. These data showed that the diagnosis of FHH cannot be ruled out by comorbidities classically associated with PHPT.

Concerning biological results, some points need to be underlined. 24-hour urinary calcium excretion in FHH patients could be within the same range as that measured in PHPT patients. In our study, despite a low 24-hour urinary calcium excretion of 2.8 mmol/24 h, CCCR ranged from 0.01 to 0.02 in 40% of patients and was even >0.02 in 10% of cases. CCCR >0.01 was reported in 5 to 35% of patients, based on published data.[3,11,12]

We did not find any correlations between age and serum calcium, which suggests that these levels remain stable on a lifelong basis around an individual set-point. This hypothesis is further evidenced by the correlation found between minimum and maximum serum calcium during follow-up. A plasma magnesium level above the

TABLE 3 Phenotypic description of patients who underwent a parathyroid surgery

	Patients with parathyroid surgery
N	14
Age at hypercalcaemia diagnosis	49 [35-55]
Age < 50 years	N = 7/13 (54%)
Total serum calcium	2.90 [2,84-2,99]
Serum calcium > 2.80 mmol/L	N = 11/14 (79%)
Osteoporosis or osteoporotic fracture	N = 2/7 (29%)
Renal colic or kidney stones at ultrasonography	N = 2/14 (14%)
Chondrocalcinosis	N = 2/12 (17%)
Cognitive impairment	N = 1/13 (8%)
Psychiatric disorders	N = 5/13 (38%)
Acute pancreatitis	N = 1/13 (8%)
24-h urinary calcium excretion (mmol/24 h)	4.1 [2.6-5.5]
24-h urinary calcium excretion >7 mmol/24 h	1/11 (9%)
Number of criteria indicative for surgery ([a])	
[a]0	1 (surgery 7 y before genetic diagnosis)
[a]1	5 (36%)
[a]2	7 (50%)
[a]3	1 (7%)

[a]Criteria for surgery: age <50 y, and/or serum calcium >2.80 mmol/L, and/or osteoporosis, and/or renal colic or kidney stones at ultrasonography.

normal range is typically described in FHH1.[13] This was not detected in our patient cohort since magnesium levels were within the normal range in 92% of patients. A normal plasma magnesium concentration was also observed by Vargas-Poussou *et al*.[7]

The French series of Vargas-Poussou *et al* highlighted an important overlap with PHPT in most of the individual laboratory values.[7] Plasma PTH concentrations were abnormally high in 23% of FHH patients. Our results are consistent with these data, with 27% of patients having high PTH levels, whereas other studies report elevated PTH values in 8 to 24% of FHH patients.[14-16]

Surgery is not routinely indicated in FHH given its ineffectiveness in normalizing calcium levels. This therapeutic choice was made independently by each medical unit. Our results showed that parathyroid surgery for a few patients with total serum calcium >3 mmol/L can reduce or even normalize serum calcium, especially if parathyroid adenoma is excised. The association between FHH1 and parathyroid adenomas is more common than anticipated from a statistical perspective. Prevalence was estimated at 2.9% in a cohort of 139 patients presenting hypercalcaemia that suggests PHPT.[17] In our study, the prevalence rate was 3.9%. Thirteen patients with FHH who underwent removal of a parathyroid adenoma are described in the literature[14,17-23]; 38% had a preoperative serum calcium above 3.2 mmol/L (Table 1).

The involvement of CaSR in PHPT pathogenesis is controversial. Nevertheless, *casr* knockout mice presented with parathyroid hypertrophy.[24] Moreover, CaSR activation stimulates a MAPK signalling pathway with an inhibitory effect on parathyroid cell proliferation. Therefore, reduced pathway activity could promote the development of an adenoma.[25] Indeed, a reduced gene and protein expression of CaSR is found in parathyroid adenomas.[26] An allelic loss at the locus of the *CASR* gene has even been identified in 10% of parathyroid adenomas in one study,[27] but not confirmed in another.[28] It has been suggested that FHH1 could predispose to the development of parathyroid adenomas.[29]

Finally, we did not find any correlation between genotype and phenotype, but only 10 mutations were reported in two or more patients. Significant inter-mutation variations were found with regard to the femoral and lumbar Z score, and plasma calcium and PTH levels in the Danish series.[3,30] However, Vargas-Poussou *et al*[7] found similar phenotypes regardless of the protein domain affected by the mutation.

4.1 | Strength and limitations of the study

The strength of this study is the high number of patients enrolled. It is one of the largest cohorts to date and is probably representative of adult FHH1 patients. Data were collected from each patient's record.

The limitations of the study are due to its retrospective design given the rarity of the pathology. Some French centres did not respond to our data request, and by consequence, the 45 patients who were followed in these centres were excluded. Moreover, 39 patients were excluded due to insufficient data in the medical files. Data were collected in 80 patients (48% of the initial population) so that it remains the largest French cohort of FHH1.

No homogeneous protocol was available for data collection, notably for the blood and urine analyses. Some data were sparse because not all parameters were investigated in each patient. In terms of laboratory data, blood and urine samples were analysed in each centre using different kits. Laboratory measurements were not standardized regarding interfering medications, time of the day or dietary intake. Nevertheless, these measurements reflect clinical practice.

Moreover, some *CASR* mutations have not been yet described and identified in terms of function. However, ACMG classification is consistent with pathogenicity. The classification of these missense variants as likely pathogenic (class 4) results from the combination of two or three moderate criteria and two supporting criteria. The distribution of the mutations, primarily in the extracellular domain of the receptor and mainly missense, is similar to that described in other studies.[7,31]

5 | CONCLUSION

The data presented here show that an atypical characteristic has been found in one-third of FHH1 patients, such as hypercalciuria,

TABLE 4 Mutation description of our study population

Localization	cDNA alteration	Protein impairment	Mutation type	Position	ACMG classification	ACMG criteria	State in the literature	Number of mutated patients
Extracellular domain	c.164C > T	p.P55L	Missense	Exon 2	5	PS3 PM1 PM2 PP2 PP3 PP5	Already reported	3
	c.197G > A	p.R66H	Missense	Exon 3	5	PS3 PM1 PM2 PM5 PP2 PP3 PP5	Already reported	1
	c.220A > G	p.M74V	Missense	Exon 3	4	PM1 PM2 PP1 PP2 PP3 PP5	Already reported	1
	c.230C > T	p.A77V	Missense	Exon 3	4	PM1 PM2 PP1 PP2 PP3 PP4 PP5	Already reported	1
	c.319G > A	p.A107T	Missense	Exon 3	4	PM1 PM2 PP2 PP3	Not described	1
	c.413C > T	p.T138M	Missense	Exon 3	5	PS3 PM1 PM2 PP2 PP3 PP5	Already reported	1
	c.419C > T	p.A140V	Missense	Exon 3	4	PM1 PM2 PP2 PP3 PP5	Already reported	4
	c.427G > C	p.G143R	Missense	Exon 3	4	PM1 PM2 PM5 PP2 PP3 PP5	Already reported	1
	c.497G > T	p.S166I	Missense	Exon 4	4	PM1 PM2 PM5 PP2 PP3	Already reported	1
	c.513C > A	p.S171R	Missense	Exon 4	4	PM1 PM2 PM5 PP2 PP3 PP5	Already reported	1
	c.514A > G	p.R172G	Missense	Exon 4	5	PS3 PM1 PM2 PP2 PP3 PP5	Already reported	1
	c.518T > C	p.L173P	Missense	Exon 4	5	PS3 PM1 PM2 PM5 PP2 PP3 PP5	Already reported	1
	c.554G > A	p.R185Q	Missense	Exon 4	5	PS3 PM1 PM2 PM5 PP2 PP3 PP5	Already reported	1
	c.568G > A	p.D190N	Missense	Exon 4	4	PM1 PM2 PM5 PP2 PP3	Not described	1
	c.593C > A	p.A198E	Missense	Exon 4	4	PM1 PM2 PP2 PP3	Not described	3
	c.652T > A	p.Y218N	Missense	Exon 4	4	PM1 PM2 PM5 PP2 PP3 PP5	Already reported	1
	c.653A > G	p.Y218C	Missense	Exon 4	5	PS3 PM1 PM2 PM5 PP2 PP3 PP5	Already reported	2
	c.658C > T	p.R220W	Missense	Exon 4	5	PS3 PM1 PM2 PM5 PP2 PP3 PP5	Already reported	1
	c.659G > A	p.R220Q	Missense	Exon 4	5	PS4 PM1 PM2 PM5 PP1 PP2 PP3 PP5	Already reported	2
	c.661C > T	p.P221S	Missense	Exon 4	5	PS3 PM1 PM2 PP2 PP3 PP5	Already reported	1
	c.679C > T	p.R227X	Nonsense	Exon 4	5	PVS1 PM1 PM2 PM4 PP1 PP5	Already reported	1

(Continues)

TABLE 4 (Continued)

Localization	cDNA alteration	Protein impairment	Mutation type	Position	ACMG classification	ACMG criteria	State in the literature	Number of mutated patients
	c. 893C > T	p.A298V	Missense	Exon 4	4	PM1 PM2 PM5 PP2 PP3	Not described	1
	c.1244G > A	p.R415Q	Missense	Exon 4	4	PM1 PM2 PP2 PP3	Not described	1
	c.1295A > G	p.Q432R	Missense	Exon 4	4	PM1 PM2 PP2 PP3 PP5	Already reported	1
	c.1377 + 1G>T	p.?	Splicing	Intron 4	5	PVS1 PM2 PP2 PP3	Not described	1
	c.1393C > T	p.R465W	Missense	Exon 5	5	PS3 PM1 PM2 PM5 PP2 PP3 PP5	Already reported	3
	c.1510G > A	p.V504M	Missense	Exon 5	4	PM1 PM2 PP2 PP5	Already reported	1
	c.1608 + 3A>C	p.?	Splicing	Intron 5	4	PM1 PM2 PP3	Already reported	1
	c.1609 −1G > T	p.?	Splicing	Intron 5	5	PVS1 PM1 PM2 PP3	Not described	1
	c.1645G > A	p.G549R	Missense	Exon 6	5	PS3 PS4 PM1 PM2 PP2 PP3 PP5	Already reported	1
	c.1661T > A	p.I554N	Missense	Exon 6	5	PS4 PM1 PM2 PP2 PP3PP5	Already reported	1
	c.1664T > C	p.I555T	Missense	Exon 6	4	PM1, PM2, PP2, PP3, PP5	Already reported	1
	c.1670G > A	p.G557E	Missense	Exon 6	4	PM1 PM2 PP2 PP3 PP5	Already reported	2
	c.1682G > C	p.C561S	Missense	Exon 6	5	PS3 PM1 PM2 PP2 PP3 PP5	Already reported	1
	c.1754G > T	p.C585F	Missense	Exon 7	4	PM1 PM2 PP2 PP3 PP5	Already reported	1
Transmembrane domain	c.1901T > C	p.F634S	Missense	Exon 7	4	PM1 PM2 PP1 PP2 PP3	Already reported	1
	c.1919C > T	p.T640I	Missense	Exon 7	4	PM1 PM2 PP2 PP4 PP5	Already reported	1
	c.1942C > T	p.R648X	Nonsense	Exon 7	5	PVS1, PM1, PM2, PP5	Already reported	2
	c.1963_1965 delCTC	p.L655del	Deletion	Exon 7	4	PM1 PM2 PM4 PP3 PP5	Already reported	1
	c.2008G > A	p.G670R	Missense	Exon 7	5	PS3 PM1 PM2 PM5 PP2 PP3 PP5	Already reported	1
	c2029dup	p.Cys677 Leufs*31	Duplication	Exon 7	5	PVS1 PM1 PM2 PM4	Not described	2
	c.2038C > A	p.R680S	Missense	Exon 7	4	PM1 PM2 PM5 PP2 PP3	Not described	1
	c.2038C > T	p.R680C	Missense	Exon 7	5	PS3 PM1 PM2 PM5 PP2 PP3 PP5	Already reported	1

(Continues)

TABLE 4 (Continued)

Localization	cDNA alteration	Protein impairment	Mutation type	Position	ACMG classification	ACMG criteria	State in the literature	Number of mutated patients
	c.2039G > A	p.R680H	Missense	Exon 7	5	PS3 PM1 PM2 PM5 PP2 PP3 PP5	Already reported	2
	c.2048C > A	p.A683D	Missense	Exon 7	4	PM1 PM2 PP2 PP3	Not described	1
	c.2072G > A	p.C691Y	Missense	Exon 7	4	PM1 PM2 PP2 PP3	Not described	1
	c.2089G > A	p.V697M	Missense	Exon 7	5	PS3 PM1 PM2 PP2 PP3 PP5	Already reported	1
	c.2083_2106dup	p.(Ile695_Val702dup)	Duplication in-frame	Exon 7	4	PM1 PM2 PM4 PP3	Not described	1
	c.2154G > A	p.W718X	Nonsense	Exon 7	5	PVS1 PM1 PM2 PM4 PP5	Already reported	1
	c.2203C > A	p.Q735K	Missense	Exon 7	4	PM1 PM2 PP2 PP3	Not described	1
	c.2243C > A	p.P748H	Missense	Exon 7	4	PM1 PM2 PM5 PP2 PP3 PP5	Already reported	1
	c.2243C > G	p.P748R	Missense	Exon 7	5	PS3 PM1 PM2 PP2 PP3 PP5	Already reported	1
	c.2386A > T	p.K796X	Nonsense	Exon 7	5	PVS1 PM1 PM2 PP5	Already reported	1
	c.2401T > C	p.F801L	Missense	Exon 7	4	PM1 PM2 PP2 PP3 PP5	Already reported	1
	c.2425T > C	p.F809L	Missense	Exon 7	5	PS1 PM1 PM2 PP1 PP2 PP3 PP5	Already reported	1
	c.2427C > G	p.F809L	Missense	Exon 7	5	PS3 PM1 PM2 PP2 PP3 PP5	Already reported	1
	c.2435T > G	p.L812R	Missense	Exon 7	4	PM1 PM2 PP2 PP3	Not described	1
	c.2467C > T	p.P823S	Missense	Exon 7	4	PM1 PM2 PP2 PP3	Not described	1
	c.2479A > G	p.S827G	Missense	Exon 7	4	PM1 PM2 PM5 PP2 PP3	Not described	1
	c.2579T > G	p.I860S	Missense	Exon 7	4	PM1 PM2 PP2 PP3 PP5	Already reported	1

Note: PVS, very strong evidence of pathogenicity; PVS1, null variant (nonsense, frameshift, canonical +/−1 or 2 splice sites, initiation codon, single or multi-exon deletion) in a gene where loss of function is a known mechanism of disease; PS, strong evidence of pathogenicity; PS1, same amino acid change as a previously established pathogenic variant regardless of nucleotide change; PS3, well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product; PS4, the prevalence of the variant in affected individuals is significantly increased compared to the prevalence in controls; PM, moderate evidence of pathogenicity; PM1, Located in a mutational hot spot and/or critical and well-established functional domain without benign variation; PM2, absent from controls (or at extremely low frequency if recessive) in gnomAD database; PM3, detected in trans with a pathogenic variant (the phase was determined); PM4, protein length changes due to in-frame deletions/insertions in a nonrepeat region or stop-loss variants; PM5, Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before; PP, supporting evidence of pathogenicity; PP1, Co-segregation with disease in multiple affected family members in a gene definitively known to cause the disease; PP2, Missense variant in a gene that has a low rate of benign missense variation and in which missense variants are a common mechanism of disease; PP3, Multiple lines of computational evidence support a deleterious effect on the gene or gene product; PP5, Reputable source recently reports variant as pathogenic but the evidence is not available to the laboratory to perform an independent evaluation.

elevated PTH plasma levels, presence of kidney stones or osteoporotic bone fracture, evidence of parathyroid adenoma on a neck ultrasonography or MIBI scintigraphy, or surgically removed parathyroid adenoma. CCCR is not sufficient to accurately distinguish between FHH from PHPT. Diagnostic criteria must be improved in order to identify FHH1 patients more effectively. Moreover, the presence of parathyroid adenomas does not appear to be sheer coincidence. Indeed, FHH patients may be more prone to developing parathyroid adenomas so that a follow-up of these patients may be useful. The classical therapeutic abstention is not necessarily warranted in all FHH1 patients. Finally, no relationship between genotype and phenotype could be identified in this large cohort of FHH type 1 patients.

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CONFLICT OF INTEREST

There is no conflict of interest to potentially prejudice the impartiality of the research reported.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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