Pro-FHH: A Risk Equation to Facilitate the Diagnosis of Parathyroid-Related Hypercalcemia

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Context: Parathyroid-related hypercalcemia is due to primary hyperparathyroidism (PHPT) or to familial hypocalciuric hypercalcemia (FHH). PHPT can lead to complications that necessitate parathyroidectomy. FHH is a rare genetic disease resembling PHPT; surgery is ineffective. A reliable method for distinguishing FHH from PHPT is needed.

Objective: To develop an easy-to-use tool to predict if a patient has PHPT.

Design: Retrospective analysis of two prospective cohorts. Development of an unsupervised risk equation (Pro-FHH).

Setting: University hospitals in Paris, France, and Aarhus, Denmark.

Participants: Patients (Paris: 65 with FHH, 85 with PHPT; Aarhus: 38 with FHH, 55 with PHPT) were adults with hypercalcemia and PTH concentration within normal range.

Main Outcome Measures: Performance of Pro-FHH to predict PHPT.

Results: Pro-FHH takes into account plasma calcium, PTH, and serum osteocalcin concentrations, and calcium-to-creatinine clearance ratio calculated from 24-hour urine collection (24h-CCCR). In the Paris cohort, area under the receiver operating characteristic curve (AUROC) of Pro-FHH was

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Abbreviations: 24h-CCCR, calcium-to-creatinine clearance ratio calculated from 24-hour urine collection; AUROC, area under the receiver operating characteristic curve; CaSR, calcium-sensing receptor; CCCR, calcium-to-creatinine clearance ratio; eGFR, estimated glomerular filtration rate; FHH, familial hypocalciuric hypercalcemia; LTF, lost to follow-up; M2, model 2; Ocn, osteocalcin; MBR, marker of bone remodeling; P_{Ca}, total calcium in plasma; P_{Cr}, total creatinine in plasma; PHPT, primary hyperparathyroidism; P_{Mg}, total magnesium in plasma; P_{Pi}, total phosphate in plasma; U_{Ca}, calcium in 24-hour urine collection sample; U_{Cr}, creatinine in 24-hour urine collection sample.

0.961, higher than that of 24h-CCCR. With a cutoff value of 0.928, Pro-FHH had 100% specificity and 100% positive predictive value for the diagnosis of PHPT; it correctly categorized 51 of 85 patients with PHPT; the remaining 34 were recommended to undergo genetic testing. No patients with FHH were wrongly categorized. In an independent cohort from Aarhus, AUROC of Pro-FHH was 0.951, higher than that of 24h-CCCR.

Conclusion: Pro-FHH effectively predicted whether a patient has PHPT. A prospective trial is necessary to assess its usefulness in a larger population and in patients with elevated PTH concentration. (*J Clin Endocrinol Metab* 103: 2534–2542, 2018)

Primary hyperparathyroidism (PHPT) is an endocrine disease with an estimated prevalence of one per 1000 in men and two per 1000 in women (1). PHPT may actually be of a higher prevalence (~1%), because of undiagnosed cases (2). The typical biological presentation of PHPT has changed over the last several decades. Currently, most patients with PHPT have mild hypercalcemia with slightly increased or even normal PTH concentration. Although PHPT is frequently asymptomatic at the time of diagnosis, it is often (in ≥50% of patients) associated with renal and/ or bone damage (3). The only cure for PHPT is para-thyroidectomy (4, 5). Recurrent laryngeal nerve injury, transient or persistent hypoparathyroidism, and/or hungry bone syndrome (6) can complicate parathyroidectomy.

Familial hypocalciuric hypercalcemia (FHH) was first reported by Foley *et al.* (7) in 1972. Its prevalence in the general population is unknown. FHH makes the diagnosis of parathyroid-related hypercalcemia more complex. FHH is an autosomal dominant disease with a high penetrance due to a defect of extracellular calcium sensing in the parathyroid glands and the kidney (8). FHH has been causally traced to loss-of-function mutations in three genes: *CASR*, encoding the calcium-sensing receptor (CaSR) in FHH1 (9, 10); *GNA11*, encoding the $G\alpha_{11}$ protein in FHH2 (11); and *AP2S1*, encoding the adaptor-related protein complex 2, sigma-2 subunit in FHH3 (12). Parathyroid gland surgery does not cure FHH and must be avoided (13).

A recent report showed that patients with PHPT and those with FHH frequently have quite similar bio-logical presentations (14): blood calcium, magnesium, and PTH concentrations, and urinary calcium excretion in patients with PHPT or FHH considerably overlap. Therefore, distinguishing between PHPT and FHH may be extremely challenging, especially when serum PTH concentration is within the normal range, as it is in up to 48% of patients with PHPT (1, 15) and ~80% of patients with FHH (14). The latest guidelines on the diagnosis of PHPT state that calcium-to-creatinine clearance ratio (CCCR) calculated from 24-hour urine collection (24h-CCCR) can help distinguish between FHH and PHPT (16): On average, urinary calcium excretion is lower in patients with FHH than in those with PHPT. As with other clinical measures in these patients, however, 24h-CCCR values overlap in FHH and PHPT.

It remains unclear whether phenotypic characteristics discriminate patients with FHH or PHPT on an individual basis. Our goal was to develop an easy-to-use tool, which we called Pro-FHH, to accurately predict whether a patient with parathyroid-related hypercalcemia has PHPT or FHH. Pro-FHH stands for "to protect FHH patients"; that is, to avoid unnecessary surgery in patients with FHH by diagnosing PHPT safely in patients with high Pro-FHH values and by performing genetic testing in all others. We studied patients with genetically proven FHH and with surgically proven PHPT and assessed the performance of Pro-FHH in two independent groups of patients with FHH or PHPT.

Patients and Methods

Development study subjects

Data were prospectively collected from January 1992 to May 2016 and analyzed from March 2015 to December 2016. Included patients met all the following inclusion criteria: (1) high, fasting, serum ionized calcium concentration (≥1.32 mM); (2) normal, fasting, concomitant PTH concentration; (3) referral to one of the inclusion centers (southwestern area of Paris: European Georges Pompidou and Cochin Hospitals). Only adults were included (≥18 years old). This study was conducted in accordance with the declaration of Helsinki and approved by the French National regulatory board (CNIL 1887067v0). All tested patients gave their informed written consent for gene analysis (Supplemental Materials). Complications (*i.e.*, nephrolithiasis, osteoporosis, and fracture) were recorded at the time of referral. The Paris cohort included 65 patients with FHH and 85 with PHPT.

Biological assessments

Treatments with loop diuretics or thiazides and calcium supplements were withdrawn prior to the study. Morning blood and urine samples were collected after an overnight fast. Ionized calcium concentration was determined in serum. In plasma, total calcium (P_{Ca}), PTH, phosphate (P_{Pi}), magnesium (P_{Mg}), 25(OH)vitamin D, osteocalcin (Ocn), and creatinine (P_{Cr}) concentrations were measured. In the second morning urine, calcium and creatinine were quantified. In urine calcium (U_{Ca}), phosphate, sodium, urea, and creatinine (U_{Cr}) concentrations in a 24-hour collection sample were quantified. Estimated glomerular filtration rate (eGFR) was estimated with the

Modification of Diet in Renal Disease formula: $186 \times (P_{Cr} \times 0.0113)^{-1.154} \times age^{-0.203}$, adapted to sex as described previously (17). Because various analytical methods for the measurement of PTH, P_{Mg}, and Ocn concentrations were used over the time (Supplemental Materials), those variables are expressed as ratio of measured value to the upper limit of normal. 24h-CCCR was calculated as previously published (18):

$$\frac{24\text{-hour } U_{Ca} \times P_{Cr}}{P_{Ca} \times 24\text{-hour } U_{Cr}}.$$

Statistical analyses

Baseline characteristics are described by median (interquartile range) or as a percentage for quantitative and qualitative data, respectively. Values were compared by Mann-Whitney or χ^2 test using Prism, version 7.0b, for MacOSX (GraphPad Software) where appropriate. For additional analyses, SAS software, version 9.4 (SAS Institute) was used. Variables were log-transformed if not normally distributed. All variables were log-transformed except age, PTH ratio, P_{Pi}, and P_{Cr}. As an exploratory study, a principal component analysis was performed to highlight similarities and/or redundancies between variables to limit the number of subsequent multivariate analyses (Supplemental Fig. 1A and 1B). A heatmap was used to visualize pairwise correlations (Supplemental Fig. 1C).

Models and risk equation

We created two different logistic regression models to predict the risk of having PHPT. Model 1 was based on the standard recommendations of care (16): the 24-hour CCCR. Model 2 (M2) was an unsupervised model: From all data, without any supervised choice, a stepwise regression selected all nonredundant variables that reached a sufficient importance ($P < 0.20$) in univariate analysis as well as age, sex, history of osteoporosis, nephrolithiasis, and fracture. Effects were entered step by step into the model when P values were <0.10 and were removed when P values were >0.05. We ensured that referral to one hospital or the other did not change the estimations of the final model. Pro-FHH, the unsupervised risk equation, was then developed using the selected variables of the multivariate model (*i.e.*, M2) as follows: $P = \frac{1}{1+e^{-X\beta}}$, where X is the vector of the selected variables and β is the vector of regression coefficients of the logistic regression.

Internal validation

Internal validation was performed by the leave-one-out cross-validation technique (Supplemental Fig. 2). We dropped data of one subject and re-estimated the parameter as many times as there were subjects. We then graphically controlled for the similarity between the cross-validated individual predicted to the individual predicted probability. We evaluated the discrimination ability by calculating the area under the receiver operating characteristic curve (AUROC) for each model. We

obtained 95% CIs after 1000 bootstrapped replications. We tested differences between each AUROC of the two models using a paired Student t test. The concordance between predicted and observed number of patients with PHPT by decile of estimated risk was evaluated by the Hosmer-Lemeshow goodness-of-fit test; $P > 0.20$ indicated an adequate calibration.

External validation

For external validation, 93 patients (55 with PHPT and 38 with FHH) from an independent, well-described (19) prospective cohort in Aarhus, Denmark, were included. They met the same inclusion criteria as patients included in the development study. Ocn was not measured in this cohort; therefore, serum alkaline phosphatase concentration as a marker of bone remodeling (MBR) was used instead, expressed as a ratio to the upper limit of the normal range. The individual probability was calculated for each of those patients, and AUROC was calculated to evaluate the capacity of the equation to discriminate correctly the patients. $P < 0.05$ was considered significant.

Results

Patient characteristics

The study flowchart is shown in Fig. 1. A total of 220 patients met the inclusion criteria, of whom 12 were lost to follow-up (LTF). A total of 116 patients were screened first for genetic abnormality; of these, 56 had FHH and 36 of the 116 patients were LTF or declined surgery. Of the 24 patients who underwent parathyroid surgery, 19
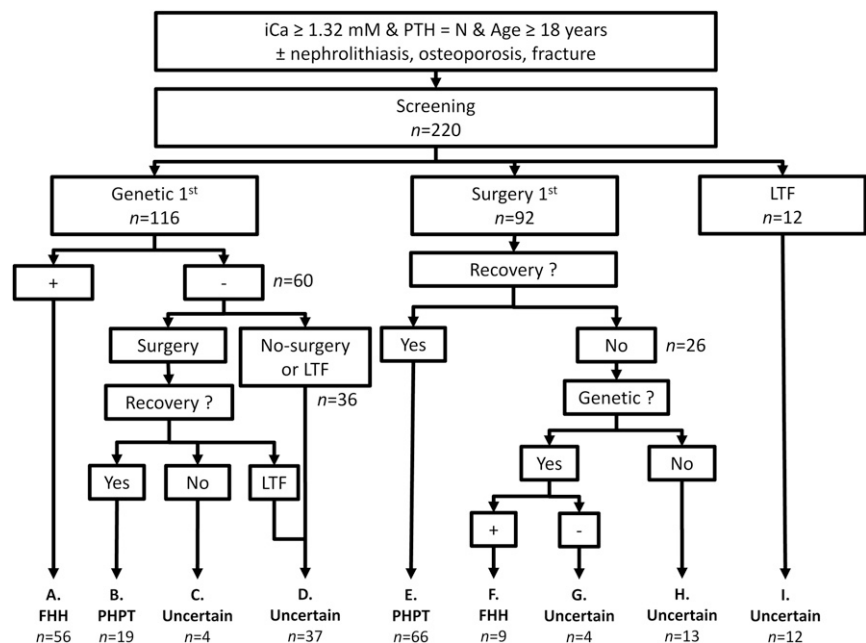

Figure 1. Flowchart of the study. A total of 220 patients met the inclusion criteria during the period of inclusion and were screened for biological and clinical history. Patients underwent genetic testing or surgery; if results were negative, they were proposed for the other arm of the study, if not LTF. Patients with FHH (n = 65) came from groups A and F, as shown in the figure; patients with PHPT (n = 85) came from groups B and E. Some patients were left with uncertain diagnosis (n = 70) because they did not recover after surgery and were negative for genetic testing (n = 8 from groups C and G), because data were missing (LTF), or because they declined surgery or genetic testing (n = 62 from groups D, H, and I). iCa, fasting ionized serum calcium concentration; LTF, lost to follow-up; N, normal.

were cured, 4 were not cured, and 1 was LTF. Ninety-two patients underwent parathyroidectomy as a first-line treatment; of those, 66 were cured and 26 were not. Of the patients who were not cured by surgery, 13 underwent genetic testing, which was positive in 9, negative in 4, and 13 had an uncertain diagnosis (*i.e.*, no genetic testing). Overall, 65 patients (30%) had genetically proven FHH (59 with FHH1, 1 with FHH2, and 5 with FHH3; gene mutations are reported in Supplemental Table 1); 85 patients (39%) had surgically proven PHPT, according the usual criteria (20); and 70 patients (32%) had uncertain diagnosis.

Diagnoses were uncertain because subjects were LTF, did not undergo genetic testing, declined surgery, or were not cured by surgery. We compared the clinical and biological characteristics of the 70 patients with uncertain diagnosis with those of included patients (*i.e.*, those with FHH or PHPT). The former were slightly older, less often had a diagnosis of osteoporosis, and had lower P_{Ca} and P_{Mg} levels and eGFR than included patients (Supplemental Table 2). In the group of patients with uncertain diagnosis, patients were older, reported more history of nephrolithiasis, and had a lower P_{Ca} but a higher 24h-CCCR than included patients with FHH. None of the other available data differed.

The clinical and biological data of patients with FHH and those with PHPT are shown in Table 1. Patients with FHH were younger than those with PHPT. Most patients (78%) were women, and this sex disequilibrium was more marked in the PHPT group than in the FHH group. At diagnosis, 32 patients (21%) reported a history of nephrolithiasis; 41 (27%), osteoporosis; and 20 (13%), a history of fracture. These findings were predominantly observed in the PHPT group. P_{Ca} concentration was higher in patients with FHH than in those with PHPT. The median PTH concentration was in the upper part of the normal range for both groups and higher in the PHPT group, as was U_{Ca} excretion [assessed on 24-hour urine collection or corrected to U_{Cr} concentration (*i.e.*, 24-hour U_{Ca}/U_{Cr} and fasting U_{Ca}/U_{Cr})], and body weight. P_{Pi} and renal threshold of phosphate (21) were in the lower part of the normal range, with no difference between groups. Daily urinary sodium and urea excretion levels were similar between patients with FHH and those with PHPT. 25(OH)vitamin D concentration was similar in both groups. The median P_{Mg} ratio was within the normal range in both groups and slightly, but significantly, higher in patients with FHH. Median MBR (Ocn) ratio was significantly higher in patients with PHPT than in those with FHH. eGFR was within the normal range and similar in both groups.

Development and assessment of performance of Pro-FHH

The principal component analysis is described in Supplemental Material and Supplemental Fig. 1. The nonredundant quantitative variables entered in the stepwise analysis were age; 24-hour U_{Cr}, fasting P_{Ca}, fasting CCCR, and 24h-CCCR levels; MBR (Ocn) ratio, P_{Mg} ratio, and PTH ratio. The qualitative variables were sex, history of fracture, nephrolithiasis, and osteoporosis. Age, PTH ratio, P_{Ca} level, MBR (Ocn) ratio, and 24h-CCCR were independently associated with the risk of having PHPT and were kept in the development of the unsupervised risk equation M2 (Table 2). Pro-FHH (*i.e.*, probability of having PHPT) was constructed as follows:

$$p = \frac{1}{1 + e^{-23.19 + 11.17 \times P_{Ca} - 7.77 \times PTH\ ratio - 3.09 \times \ Ln(24h - CCCR) - 2.89 \times \ Ln(MBR\ ratio)}}$$

We compared Pro-FHH performance to predict PHPT with the currently recommended criterion for diagnosis, which relies only on 24h-CCCR (Fig. 2). The AUROC of Pro-FHH was significantly higher [0.961 (0.960 to 0.962)] than that of 24h-CCCR [0.862 (0.844 to 0.862), $P < 0.001$]. Using cutoff values of 0.928 and 2% for Pro-FHH and 24h-CCCR, respectively, no patient with FHH was incorrectly categorized as having PHPT by Pro-FHH and two patients with FHH (10%) were incorrectly categorized as having PHPT by 24h-CCCR (Table 3). The specificity and the positive predictive value of Pro-FHH for the diagnosis of PHPT were both 100%. Using cutoff values of 0.062 and 1% for Pro-FHH and 24h-CCCR, respectively, no patient with PHPT was incorrectly categorized as having FHH by Pro-FHH, but 22 patients with PHPT were incorrectly categorized as having FHH by 24h-CCCR (Table 3). The specificity and the positive predictive value of Pro-FHH for the diagnosis of FHH were both 100%.

External validation of Pro-FHH

Patients from the independent cohort were 61 (51 to 72) years old, and median 24h-CCCR was 1.20 (0.70 to 1.90). AUROC of Pro-FHH was significantly higher [0.951 (0.950 to 0.952)] than that of 24h-CCCR [0.878 (0.877 to 0.881), $P < 0.001$]. Using cutoff values of 0.928 and 2% for Pro-FHH and 24h-CCCR, respectively, no patient with FHH was incorrectly categorized as having PHPT by Pro-FHH or by 24h-CCCR. The specificity and the positive predictive value of Pro-FHH for the diagnosis of PHPT were both 100%. Using cutoff values of 0.062 and 1% for Pro-FHH and 24h-CCCR, respectively, five patients with PHPT (13%) were incorrectly categorized as having FHH by Pro-FHH, and eight patients with PHPT (21%) were incorrectly categorized as having FHH by 24h-CCCR (Table 3). The

Table 1.   Characteristics of Patients Included in the Development Study

	FHH (n = 65)	PHPT (n = 85)	*P*
Age, y	49 (39–62)	59 (52–68)	<0.001
Women, no. (%)	44 (68)	73 (86)	0.01
Postmenopausal women, no. (%)	26 (59)	58 (79)	0.02
BMI, kg/m^2	24.6 (21.3–27.5)	23.4 (21.5–26.5)	0.31
History of nephrolithiasis, no. (%)	8 (12)	24 (28)	0.02
History of osteoporosis, no. (%)	8 (12)	33 (35)	0.001
History of fracture, no. (%)	4 (6)	16 (19)	0.02
Basal biology			
Fasting plasma calcium, mM	2.62 (2.54–2.71)	2.53 (2.46–2.58)	<0.001
Fasting ionized calcium, mM	1.41 (1.37–1.47)	1.37 (1.34–1.41)	<0.001
Fasting serum PTH ratio[a]	0.70 (0.55–0.87)	0.86 (0.75–0.91)	<0.001
Fasting U_{Ca}/U_{Cr}, mmol/mmol	0.15 (0.10–0.25)	0.45 (0.32–0.65)	<0.001
Fasting plasma phosphate, mM	0.86 (0.75–0.96)	0.86 (0.76–0.96)	0.72
TmPi/GFR, mmol/L GF	0.83 (0.70–0.98)	0.80 (0.68–0.89)	0.24
Urinary creatinine excretion, mmol/d	10.60 (8.60–13.00)	9.50 (8.40–11.15)	0.08
Urinary phosphate excretion, mmol/d	20.9 (17.1–25.6)	22.0 (18.5–27.9)	0.21
Urinary calcium excretion, mmol/d	2.24 (1.20–3.67)	4.86 (3.69–7.23)	<0.001
Urinary sodium excretion, mmol/d	124 (92–158)	132 (96–167)	0.47
Urinary urea excretion, mmol/d	319 (223–407)	301 (240–366)	0.66
Plasma 25(OH)vitamin D, nM	59 (42–88)	67 (45–84)	0.78
Fasting plasma magnesium ratio[a]	0.90 (0.84–0.97)	0.86 (0.80–0.88)	<0.001
Plasma osteocalcin ratio[a]	0.81 (0.62–1.07)	1.24 (0.97–1.64)	<0.001
Plasma creatinine, μM	74 (61–85)	68 (58–76)	0.003
eGFR, mL/min/1.73 m^{2b}	85 (70–109)	88 (77–106)	0.42
Fasting CCCR, %	0.43 (0.26–0.73)	1.17 (0.89–1.53)	<0.001
24h-CCCR, %	0.56 (0.34–0.87)	1.34 (0.99–1.82)	<0.001
U_{Ca}/body weight, mmol/kg	0.03 (0.02–0.06)	0.08 (0.06–0.12)	<0.001
24-hour U_{Ca}/U_{Cr}, mmol/mmol	0.21 (0.12–0.33)	0.51 (0.38–0.73)	<0.001

Data are given as median (interquartile range), unless otherwise indicated.

Abbreviations: BMI, body mass index; GF, glomerular filtrate; GFR, glomerular filtration rate; TmPi, renal threshold for phosphate.

[a]More than one type of assay was used, and data are expressed as the ratio of measured value to upper limit of normal.

[b]By Modification of Diet in Renal Disease formula.

specificity and the positive predictive value of Pro-FHH for the diagnosis of FHH were both 100%. Overall, 24h-CCCR categorized 40 patients (17% of total study population) in the PHPT group (of whom two had FHH). Pro-FHH categorized significantly (*P* = 0.01) more patients (n = 60; 25% of total study population) in the PHPT group; none was misdiagnosed (Fig. 3).

Discussion

Parathyroid surgery is the only means to cure PHPT. Parathyroidectomy can fail to cure PHPT (4), however,

and can be complicated (6). Parathyroidectomy must not be performed in patients with FHH. Because it is challenging to distinguish FHH from PHPT when plasma PTH concentration lies within the normal range, we focused on this population. An option is to perform genetic testing in all patients with hypercalcemia who have a normal PTH level, because "normocalcemic" FHH does exist but seems to be extremely rare (22). This, however, would increase significantly the number of tests performed, increasing costs, overloading medical genetic departments, and delaying the time to surgery for patients with PHPT. In addition, it is likely that one or more

Table 2.   Characteristics and Performances of the Two Models

Model	Variables	No.	OR (95%CI)	AUROC	AUROC Bootstrap	HL-χ^2
1: Recommendations	Ln(24h-CCCR) per 0.1	148[a]	1.32 (1.20–1.45)	0.862	0.862 (0.860–0.844)	0.26
2: Unsupervised (*i.e.*, Pro-FHH)	P$_{Ca}$ per 0.01	147[a]	0.89 (0.84–0.96)	0.961	0.961 (0.960–0.962)	0.90
	PTH ratio per 0.1		2.17 (1.42–3.34)			
	Ln(24h-CCCR) per 0.1		1.36 (1.21–1.53)			
	Ln(MBR ratio) per 0.1		1.33 (1.14–1.56)			

Abbreviation: Ln, natural log.

[a]Due to missing data, the number of patients is lower than the number included in the study.
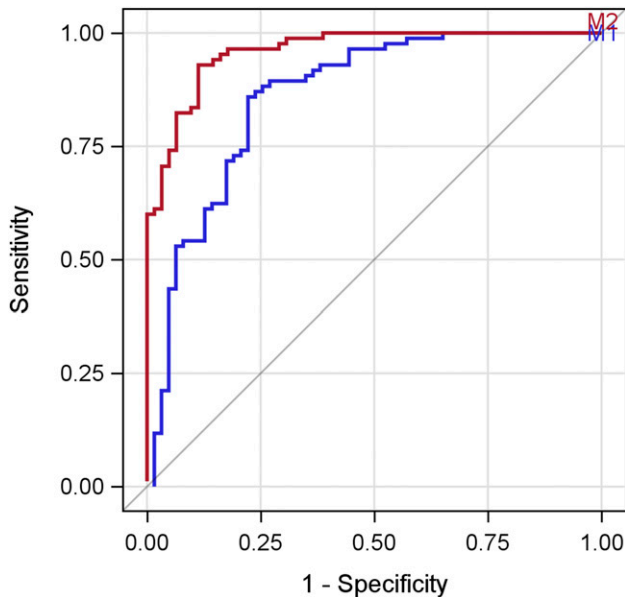
Figure 2. AUROC curves of 24h-CCCR and Pro-FHH. In the development study, model 1 was based on standard recommendations of care (16): the 24h-CCCR. M2 was the unsupervised Pro-FHH. The AUROC of M2 (Pro-FHH, red curve) is significantly (P < 0.0001) higher than that of model 1 (24h-CCCR, blue curve).

genes causing FHH are currently unknown and, therefore, a negative genetic test might not be able to rule out FHH (14, 23). Moreover, genetic analyses could find variants of uncertain pathogenicity; here, we applied the recommendations by the international guidelines (24), even if there is no definitive substitute to functional analysis. We chose to develop a statistical test to reliably predict whether a patient with hypercalcemia has PHPT, which obviates the need for genetic testing.

In development of the risk assessment, we analyzed patients with FHH and with PHPT resembling FHH because plasma PTH level was within the normal range. Seventy patients LTF or with uncertain diagnoses could not be included in the study, and because the patients who were not included had 24h-CCCR, plasma PTH, and MBR values similar to those of included patients, it is unlikely that the main conclusions of this study would have been different if these patients had been included. Data from this group were closer to those of patients with PHPT than to those of patients with FHH, suggesting that the prevalence of PHPT in nonincluded patients was higher than that of FHH.

FHH was previously reported to be due to a mutation of the *CASR* gene in two thirds of patients (25). In our cohort, the percentage was higher. About 90% of patients in our cohorts had FHH1, as recently published (14). The results should not have been different if the distribution of different FHH subtypes had been different, because Pro-FHH values do not differ between FHH type 1 and 3. PHPT is complicated by nephrolithiasis,

osteoporosis, and fractures in 36%, 63%, and 14% of patients, respectively (3). In our cohort, the prevalence of complications seems similar or slightly lower, maybe due to a less severe disease, because the PTH level was within the normal range.

On average, clinical (*i.e.*, age, sex, and medical history) and biological (*i.e.*, P_{Ca}, U_{Ca}, P_{Mg}, PTH, and Ocn) data significantly differ between the FHH and PHPT groups. However, the individual performance of those variables to distinguish FHH from PHPT is clinically insufficient because values greatly overlap between groups. A familial history of hypercalcemia could be helpful for the diagnosis of FHH, because this disease is inherited as an autosomal dominant trait; however, a familial history of hypercalcemia was known in only 24 of patients with FHH (37%) in our cohort. In addition, mutations could also occur *de novo*, meaning that there would be no familial history of hypercalcemia. Moreover, familial PHPT inherited as an autosomal dominant trait also exists [*e.g.*, type 1 multiple endocrine neoplasia (26) or hyperparathyroidism-jaw tumor (27) syndromes and isolated familial PHPT]. Further complicating diagnosis, FHH and PHPT (28) or FHH and multiple endocrine neoplasia (29) may coexist in the same patients; it was not the case in our cohort. The personal history of normal P_{Ca} is relevant: New onset of hypercalcemia indicates PHPT, whereas patients with FHH have a lifelong hypercalcemia. In most patients with PHPT in this study, however, there was no previous analysis of P_{Ca}. P_{Mg} concentration and 24-hour magnesium-to-creatinine clearance ratio differ between patients with FHH and those with PHPT (30); like 24h-CCCR, the 24-hour magnesium-to-creatinine clearance ratio is lower in patients with FHH than in those with PHPT. Unfortunately, due to the retrospective design, 24-hour urinary magnesium excretion was not measured in most patients in this study. Even if P_{Mg} was significantly higher in the FHH group in our cohort, multivariate analysis did not identify P_{Mg} as an independent variable useful to distinguish patients with FHH and those with PHPT. Moreover, in previous studies, the relations between plasma and urinary magnesium in patients with FHH or PHPT differed less than the relations between P_{Mg} and U_{Ca} (14, 30).

The latest guidelines recommend that 24h-CCCR be used to distinguish FHH from PHPT: A low 24h-CCCR (<1%) favors FHH, whereas high 24h-CCCR (>2%) favors PHPT (16). These cutoff values were determined on the basis of the earliest and the latest (18, 30, 31) systematic comparison studies between patients with FHH and patients with PHPT. Because 24h-CCCR can be higher than 2% in patients with FHH and lower than 1% in those with PHPT (18), this measure cannot reliably

Table 3.   Categorization of Patients According to 24h-CCCR and Pro-FHH

	No. of Patients With FHH	No. of Patients With PHPT	Total No.	Positive Predictive Value[a], %	Negative Predictive Value, %	Sensitivity, %	Specificity[a], %
Performance in diagnosis of PHPT							
24h-CCCR in the development cohort							
≤2%	61	67	128				
>2%	2	18	20	90.0	47.7	21.2	96.8
Total	63[b]	85	148				
p-Pro-FHH[c] in the development cohort							
≤0.928	62	34	96				
>0.928	0	51	51	100.0	64.6	60.0	100.0
Total	62[b]	85	147				
24h-CCCR in validation cohort							
≤2%	38	35	73				
>2%	0	20	20	100.0	52.0	36.4	100.0
Total	38	55	93				
p-Pro-FHH in validation cohort							
≤0.928	38	46	84				
>0.928	0	9	9	100.0	45.2	16.4	100.0
Total	38	55	93				
Performance in diagnosis of FHH							
24h-CCCR in the development cohort							
<1%	50	22	72	69.4	82.9	79.4	74.1
≥1%	13	63	76				
Total	63[b]	85	148				
p-Pro-FHH in the development cohort							
<0.062	38	0	38	100.0	78.0	61.3	100.0
≥0.062	24	85	109				
Total	62[b]	85	147				
24h-CCCR in validation cohort							
<1%	30	8	38	78.9	85.4	78.9	85.4
≥1%	8	47	55				
Total	38	55	93				
p-Pro-FHH in validation cohort							
<0.062	33	5	38	86.8	90.9	86.8	90.9
≥0.062	5	50	55				
Total	38	55	93				

[a]For Pro-FHH, positive predictive value and specificity are 100% at the cutoff values of 0.062 and 0.928, respectively. The standard recommendations of care for diagnosis, 24h-CCCR, misclassified 24 patients (17%). In the validation cohort, Pro-FHH equation misclassified five patients (5.4%), whereas the standard misclassified eight patients (8.6%).

[b]Due to missing data, the number of patients is lower than the number of included in the study.

[c]The probability of PHPT yielded by the unsupervised pro-FHH equation.

distinguish patients with FHH from those with PHPT. In the present study, two patients with FHH (2%) had 24h-CCCR values >2%, and 22 patients with PHPT (26%) had 24h-CCCR values <1%. Deficiency in 25(OH)vitamin D, which can decrease 24h-CCCR (32), was rare in our cohort: Only eight patients (5%) had a concentration <25 nM, and deficiency frequency was similar between FHH and PHPT groups and unlikely to explain the only fair performance of 24h-CCCR. Moreover, because the glomerular filtration rate is included in the calculation of 24h-CCCR, renal insufficiency could affect 24h-CCCR. In our cohorts, most patients had normal eGFR, and no difference was seen between groups. Therefore, the caveats of 24h-CCCR are likely more intrinsic than extrinsic. The difference in 24h-CCCR between patients with FHH and the patients with PHPT mainly reflects the different renal handling of calcium, because renal tubular expression of CaSR is activated in the latter and inactivated in the former. However, bone remodeling also differs; as previously reported by others (33), patients with PHPT have higher bone turnover than patients with FHH. Therefore, we included a MBR (specifically, Ocn in the French cohort, and alkaline phosphatase in the Danish cohort, depending on the available data) into the risk equation. Doing so increases the diagnostic performance of Pro-FHH over that of 24h-CCCR. Bone remodeling is increased in postmenopausal women; therefore, menopausal status could have affected the performance of Pro-FHH. Actually, the circulating level of Ocn and alkaline phosphatase was slightly higher in postmenopausal than in premenopausal women, but this did not affect the performance of Pro-FHH (data not shown).
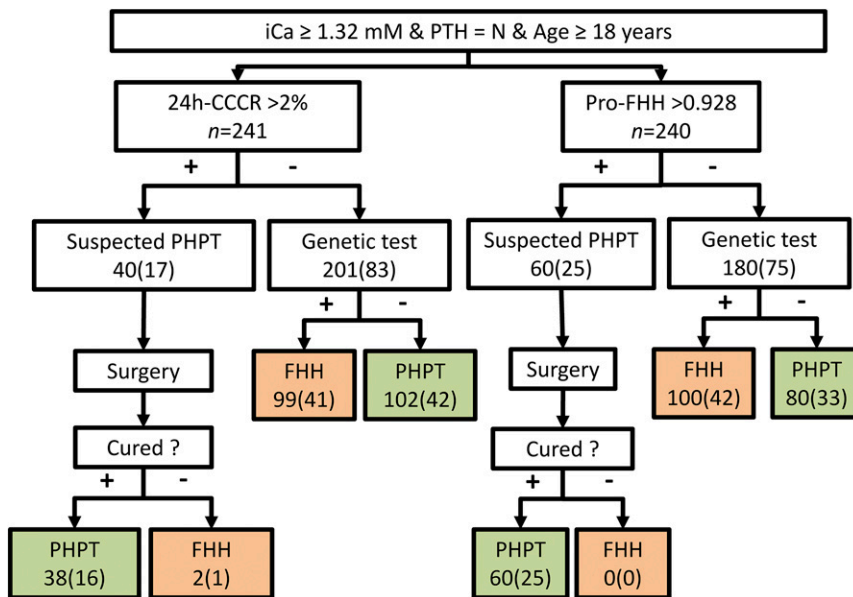
Figure 3. Performances of 24h-CCCR and Pro-FHH. In merged cohorts (from Paris and Aarhus), when 24h-CCCR was applied, PHPT was suspected in 17% of patients, two were misdiagnosed (FHH), and 83% had to be tested for FHH genes. When Pro-FHH was applied, PHPT was suspected in 25% of patients, none were misdiagnosed, and 75% had to be tested for FHH genes. iCa, fasting ionized serum calcium concentration; N, normal.

The development of Pro-FHH suffers some weaknesses. First, its development was based on retrospective design, because our patients were not systematically tested. Due to this design (and the scarcity of FHH), some assays changed over the period of inclusion (*e.g.*, those for P_{Mg}, Ocn, and PTH); therefore, we expressed the results as a ratio to the upper limit of the normal range. Our findings should now be tested in an independent cohort and a same assay used throughout. Second, only patients having a PTH level within the normal range were included. Hyperparathyroidism occurs less frequently in patients with FHH than in patients with PHPT (*i.e.*, only 20% of patients with FHH (14) and >50% of those with PHPT have hyperparathyroidism), it is a less commonly questioning presentation. Whether Pro-FHH is valid in patients with high PTH values remains unknown and must be now assessed; the prevalence of PHPT should be higher again in this population; therefore, the performances of Pro-FHH (to diagnose PHPT with a cutoff value >0.928) should not be affected. Strengths are that data were collected from expert centers and analyzed without any supervision, and that the performance of Pro-FHH was validated in an independent cohort. Moreover, Pro-FHH has been made as simple as possible and could be easily adopted by all practitioners because it requires only a measure of calcium, creatinine (in blood and urine), PTH, and a BMR.

In an era in which PHPT is underdiagnosed (2), our machine-learning (34) approach resulted in a tool that should increase the diagnosis of PHPT without risk of confusion between FHH and PHPT. In our cohorts, the use of Pro-FHH instead of 24h-CCCR would have spared unnecessary surgery in two of 100 patients with FHH and unnecessary genetic testing in 21 of 140 patients with PHPT. Pro-FHH had higher AUROC (~0.95) compared with 24h-CCCR (~0.80) and 100% specificity and 100% positive predictive value for the diagnosis of PHPT. Therefore, use of Pro-FHH will result in a better safety profile and will spare time and money compared with the current standard.

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