CLINICAL STUDY

Plasma 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D, and parathyroid hormone in familial hypocalciuric hypercalcemia and primary hyperparathyroidism

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Abstract

Introduction: Familial hypocalciuric hypercalcemia (FHH) is a lifelong, benign, inherited condition caused by inactivating mutations in the calcium-sensing receptor (CASR) gene. Both FHH and primary hyperparathyroidism (PHPT) are characterized by elevated P-calcium, normal or elevated plasma-parathyroid hormone (P-PTH), and typically normal renal function. In PHPT, vitamin D metabolism is typically characterized by low plasma levels of 25-hydroxyvitamin D (25OHD), and high plasma levels of 1,25-dihydroxyvitamin D (1,25(OH)₂D). In FHH, the vitamin D metabolism is not very well known. **Objective:** To compare and evaluate plasma 25OHD, 1,25(OH)₂D, and PTH in FHH and PHPT.

Design: Cross-sectional study.

Materials: About 66 FHH patients with mutations in the CASR gene, 147 patients with surgically verified PHPT, and 46 controls matched to FHH patients according to age (± 5 years), sex, and season. All patients had a P-creatinine $< 140 \mu\text{mol/l}$.

Methods: We measured P-calcium, P-Ca²⁺, P-albumin, P-creatinine, P-phosphate, P-magnesium, and P-PTH by standard laboratory methods. P-25OHD and P-1,25(OH)₂D were measured by RIA or enzyme immunoassay. In FHH, all protein-coding exons in the CASR gene were sequenced and aligned to GenBank reference sequence NM_000388.2.

Results: PHPT patients had higher body mass index ($2p < 0.01$), together with higher P-PTH ($2p < 0.01$) and P-1,25(OH)₂D ($2p < 0.01$) compared with FHH patients. The groups had similar levels of P-Ca²⁺ and of P-25OHD. The phenotypic expression of the CASR mutations (as determined by the degree of hypercalcemia) did not influence the levels of P-1,25(OH)₂D.

Conclusion: Even though P-calcium and P-25OHD were comparable, P-1,25(OH)₂D and P-PTH differed between FHH and PHPT.

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Introduction

Familial hypocalciuric hypercalcemia (FHH, OMIM# 145980) is an autosomal dominant condition caused by heterozygous, inactivating mutations in the calcium-sensing receptor (CASR) gene (1, 2). The CASR regulates blood calcium levels within a narrow range. It is located on the cell membrane of many different cells, but regulates plasma calcium homeostasis mainly through its expression in the parathyroid glands and the kidney tubules (3). At least 64 inactivating mutations have previously been described in the CASR gene (4). With respect to the present population of FHH patients, we have recently described 22 inactivating mutations, of which 19 were previously unpublished (5).

FHH and primary hyperparathyroidism (PHPT) are characterized by elevated plasma calcium, normal or elevated plasma-parathyroid hormone (P-PTH), and

typically normal renal function. The complexity of vitamin D metabolism in PHPT has previously been described in detail (6–8). In short, plasma 25-hydroxyvitamin D (25OHD) is characteristically reduced independently of season, sex, or age (6). The excess PTH and the hypophosphatemia increase the renal production of 1,25-dihydroxyvitamin D (1,25(OH)₂D) (7). This effect appears to be mitigated by the hypercalcemia (9, 10) and possibly by increased plasma levels of fibroblast growth factor-23 (FGF23) (11, 12). The increase in 1,25(OH)₂D levels will *per se* enhance the inactivation of 25OHD and 1,25(OH)₂D through renal 24-hydroxylation (9). The hypercalcemia and the elevated plasma 1,25(OH)₂D levels could reduce dermal vitamin D production through effects on keratinocytes and melanocytes (13, 14). Whether the effects of plasma Ca²⁺ on dermal cells and renal 1 α -hydroxylase are mediated via the CASR is at present unknown. The increased body mass index (BMI)

in PHPT (15) may contribute to a reduction in plasma 25OHD and 1,25(OH)₂D (16). Finally, a pre-existing vitamin D insufficiency could theoretically predispose to the development of PHPT (6). Vitamin D metabolism has been explored only in a small number of FHH patients (17–19). We have recently reported on the ability of the calcium/creatinine clearance ratio (CCCR) and other variables of renal calcium excretion to discriminate between FHH and PHPT (20), as described by Marx *et al.* and others (1, 21–26).

The aims of the present study:

- To describe vitamin D metabolites in FHH compared with normal reference levels and healthy controls.
- To explore relations between vitamin D metabolites and other clinical variables in FHH.
- To compare vitamin D metabolites and plasma PTH levels in FHH with the levels in PHPT.

Design

We used a cross-sectional design. All patients were subjected to a standardized series of investigations (20). In PHPT, the diagnostic gold standard was histopathological findings at neck exploration leading to normocalcemia 2 months after surgery. In FHH patients, the diagnostic gold standard was the confirmation of a clinically significant mutation (i.e., resulting in hypercalcemia) in the *CASR* gene (5, 20). The more frequent polymorphisms, which may also be found in normocalcemic individuals, are not considered in this study (5).

Patients and materials

The study was carried out at 56°N and covered all seasons apart from September for FHH patients. From August 2003 to June 2007, we included 66 FHH patients (44 females and 22 males, aged 17–83 years) from 25 kindreds. One FHH patient had one parathyroid gland removed by surgery, and continued to be hypercalcemic. We compared the FHH patients with 147 hypercalcemic PHPT patients (116 females and 31 males, aged 13–86 years) diagnosed by neck surgery between April 2003 and February 2007. All PHPT patients had elevated or high normal plasma PTH. The upper third of the normal reference range was included, since plasma PTH depends on the vitamin D status in the reference population (27, 28). After parathyroid surgery, histopathological examination revealed adenoma(s) in 119 of the patients, hyperplasia in 26, and combined adenoma and hyperplasia in 2 patients.

Exclusion criteria for both patient groups included reduced renal function (plasma creatinine > 140 µmol/l), other calcium-metabolic or bone diseases, lithium treatment, systemic glucocorticoid treatment for more

than 6 months, malignant disease, uncontrolled or newly diagnosed chronic disease, and hospital admission due to drug or alcohol abuse.

As normal controls for vitamin D metabolites, we used baseline data from healthy men and postmenopausal women randomly selected from the local background population and used in previous physiological studies carried out between 1998 and 2002 (29–31). Normal subjects with illnesses or medications known to affect bone mineral metabolism (including hormone replacement therapy, diuretics, and pharmacological doses of calcium and vitamin D supplements) were excluded. Other biochemical variables were compared with our normal laboratory reference values.

We could match 46 of the normal controls to the FHH patients according to age (± 5 years), sex, and season (summer/winter).

The study was approved by the Regional Ethical Committee (Aarhus County #20030195). All patients with FHH gave informed verbal and written consent. The database was approved by the Danish Data Protection Agency.

Methods

Biochemistry

We measured plasma and urine calcium, plasma Ca²⁺, albumin, creatinine, and phosphate by standard laboratory methods. We corrected total plasma calcium for individual variations in albumin using the equation: adjusted plasma calcium (mmol/l) = plasma calcium total (mmol/l) + 0.00086 × (650 – plasma albumin (µmol/l)). We measured plasma intact PTH using the Elecsys 2010 immunoassay (Roche Diagnostics). The inter- and intra-assay coefficients of variation (CV) were less than 6 and 2% respectively.

We measured plasma 25OHD by enzyme immunoassay (IDS, Phoenix, AZ, USA). The intra- and inter-assay CV were 5–7% (concentration level: 39–165 nmol/l) and 5–9% (concentration level: 40–132 nmol/l) respectively. However, in our normal controls, 25-OHD was measured using an equilibrium RIA (DiaSorin Inc., Stillwater, MN, USA) with inter- and intra-assay CV values of 13 and 10% respectively. In order to be comparable with the IDS method, the DiaSorin results were adjusted by the formula: $Y \text{ (nmol/l)} = 1.23 X \text{ (nmol/l)} + 4.7$ based on double measurements in 123 patients (32).

We measured plasma 1,25(OH)₂D by RIA (IDS) with the intra- and inter-assay CV from 9 to 11% (concentration level: 37–113 pmol/l) and 10 to 14% (concentration level: 21–135 pmol/l) respectively.

In our normal controls, we measured plasma 1,25(OH)₂D by the RIA (Nichols Institute, San Clemente, CA, USA) with inter- and intra-assay CV values, both of 11%. In order to be comparable with the IDS method, the Nichols results were adjusted by the formula: Y

(pmol/l) = 1.138 $\times$ (pmol/l) + 3.9, based on double measurements in 74 patients (32). We constructed normal 95% confidence intervals for these variables.

The CCCR was calculated as: CCCR = (24 h-U-calcium/P-calcium, total)/(24 h-U-creatinine/P-creatinine) with variables entered as mmol or mmol/l.

All analyses were performed in non-fasting individuals.

Genetics

We characterized FHH patients by molecular genetic analysis of the *CASR* gene. This method has previously been described in detail (5). Briefly, all exons, including a minimum of 10 bp of flanking intron sequence, were sequenced directly following PCR and clean-up of PCR products, using BigDye Terminator chemistry (Applied Biosystems, Foster City, CA, USA). Sequences were aligned to GenBank reference sequence NM_000388.2. Findings were confirmed in a second analysis on separately drawn blood. The *CASR* mutations in the present FHH group have previously been published (5, 20).

Statistical analysis

We expressed basic variables by their median and range, or mean $\pm$ s.d. We assessed between group differences in means by *t*-test after logarithmic conversion when necessary. Differences between frequencies were assessed by Fisher's exact test. We used multiple linear regressions to adjust for covariates with a significance

level for entry of $P \leq 0.05$, and for removal of $P > 0.10$. Adjusted values were compared using the *F*-test. Statistical comparisons that included inter-mutation variation were performed using the one-way ANOVA. Statistical analyses were performed with SPSS version 15.0 software (SPSS Inc., Chicago, IL, USA). All *P* values mentioned in this paper are two-sided, and the cut-off value of significance is 0.05.

Results

FHH patients: baseline characteristics

The clinical characteristics of the 66 FHH patients are described in detail in Table 1 and compared with normal reference values. All were hypercalcemic, 15 (23%) had elevated P-PTH, and 16 (24%) had reduced plasma phosphate. A total of 52 (93%) had a renal calcium excretion < 7 mmol/24 h. The CCCR varied between 0.002 and 0.026 (mean = 0.008). There were no larger abnormalities in plasma creatinine (P-creatinine < 140 μ mol/l) or creatinine clearance rate. Plasma levels of 25OHD and 1,25(OH)₂D metabolites were mostly within reference limits for normal sex-, age-, and season-matched controls. The 25OHD did not differ statistically between the matched FHH ($N=46$) and controls ($N=46$), $2p=0.37$. The matched FHH group ($N=46$) had a significantly higher level of 1,25(OH)₂D (131 ± 45 (s.d.), pmol/l) than the controls (mean = 93 ± 40 (s.d.), pmol/l), $2p < 0.01$. In 54 out of the 66 FHH patients, the baseline characteristics have previously been published (20).

Table 1 Clinical characteristics of 66 patients with familial hypocalciuric hypercalcemia. Biochemical values are compared with normal laboratory reference values.

FHH	N	Median (range) or %	95% Reference values	No. of subjects with values outside 95% reference values
Age (years)	66	50 (17–83)		
Females (%)	66	66.7		
Height (m)	66	1.68 (1.52–1.90)		
Body weight (kg)	66	68.8 (50.1–134.5)		
BMI (kg/m ²)	66	23.8 (18.2–39.3)		
P-Calcium ionized (mmol/l)	66	1.41 (1.32–1.70)	1.15–1.30	66 [i]
P-Calcium total (mmol/l)	66	2.66 (2.46–3.09)	2.20–2.55	57 [i]
P-Calcium alb. adj. (mmol/l)	66	2.69 (2.54–3.16)	2.20–2.52	66 [i]
P-Creatinine (μ mol/l)	66	70 (38–132)	44–115	1 [d], 1 [i]
P-PTH (pmol/l)	66	5.3 (2.5–20.8)	1.6–6.9	15 [i]
P-Phosphate (mmol/l)	66	0.90 (0.49–1.39)	0.80–1.50	16 [d]
P-25OHD (nmol/l)	66	57 (18–154)	18–110 ^a	6 [i]
Summer (April–Sept)	26	74.5 (35–154)	43–110 ^a	2 [d], 3 [i]
Winter (Oct–March)	40	50 (18–127)	18–85 ^a	4 [i]
P-1,25(OH) ₂ D (pmol/l)	66	126 (56–307)	39–220 ^a	2 [i]
Creatinine clearance (ml/min)	56	108 (51–194)	70–140	4 [d], 8 [i]
24H-U-Calcium (mmol)	56	2.7 (0.7–9.5)		
♂	17	2.9 (1.1–7.1)	♂: 2.0–9.0	3 [d], 0 [i]
♀	39	2.6 (0.7–9.5)	♀: 2.0–7.0	10 [d], 3 [i]
Calcium/creatinine clearance ratio	56	0.006 (0.002–0.026)	–	

[i] Increased values. [d] Decreased values.

^aReference values are based on 46 healthy controls matched to FHH patients with respect to age (± 5 years), sex, and season, and collected during summer ($N=21$) and winter ($N=25$) periods.

FHH patients: determinants of vitamin D metabolites and PTH

Table 2 summarizes correlations of vitamin D metabolites and PTH in FHH with other clinical variables using adjusted multiple regression analysis (partial correlation coefficients) after adjustment for age, sex, and BMI.

Plasma PTH showed positive partial correlations with plasma levels of total calcium ($r_p=0.30$, $P=0.02$), albumin-adjusted calcium ($r_p=0.39$, $P<0.01$), Ca^{2+} ($r_p=0.34$, $P<0.01$), and creatinine ($r_p=0.34$, $P<0.01$), and correlated inversely with creatinine clearance rate ($r_p=-0.47$, $P<0.02$). Contrary to the findings in PHPT, plasma PTH was not related to plasma 25OHD in FHH (Fig. 1). Moreover, PTH was not related to age, sex, plasma 25OHD, and phosphate in a backward model ($N=66$) that also included BMI ($r_p=0.33$, $P<0.01$), Ca^{2+} ($r_p=0.46$, $P<0.01$), creatinine ($r_p=0.46$, $P<0.01$), and $1,25(OH)_2D$ ($r_p=0.31$, $P=0.02$).

Plasma 25OHD varied significantly ($P<0.01$) with season with the highest levels during summer (Fig. 2). Following adjustment for sex, age, and BMI, there was a trend toward inverse correlations to the plasma calcium variables and positive correlations to plasma levels of phosphate and creatinine (Table 2). Plasma 25OHD did not correlate with the renal 24-h calcium excretion or CCCR.

Plasma $1,25(OH)_2D$ showed no seasonal variation. Following adjustment for sex, age, and BMI, it showed no correlation to other biochemical variables (Table 2). However, in a backward model also including plasma levels of Ca^{2+} , creatinine, PTH, and phosphate, the plasma $1,25(OH)_2D$ correlated inversely to plasma Ca^{2+} ($r_p=-0.29$, $P=0.02$) and positively to plasma PTH ($r_p=0.28$, $P=0.02$) with a trend toward an inverse correlation with plasma creatinine ($r_p=-0.23$, $P=0.07$).

Phenotypic expression of CASR mutation (as expressed by the degree of hypercalcemia): Fig. 3 shows plasma $1,25(OH)_2D$ levels in the 14 different

CASR mutations included in this paper. $1,25(OH)_2D$ shows no significant inter-mutation variation (one-way ANOVA, $P=0.96$). We have previously demonstrated that plasma levels of PTH (one-way ANOVA, $P<0.01$) and plasma Ca^{2+} (one-way ANOVA, $P<0.01$) differ significantly between the different mutations (5).

Differences between FHH and PHPT

Table 3 compares descriptive and biochemical variables between FHH and PHPT patients. The FHH patients were younger, taller, and had a lower body weight than the PHPT patients. Plasma Ca^{2+} levels were identical, but total and albumin-adjusted calcium levels were slightly higher in the PHPT patients. PHPT patients had higher plasma creatinine levels and lower renal clearance rates than the FHH patients. Plasma PTH was increased by two- to threefold in the PHPT patients. Only 15 out of the 66 FHH patients (23%) had elevated plasma PTH compared with 133 out of the 147 PHPT patients (90%), $2p<0.01$. As to be expected, the FHH group had a significantly lower average urinary calcium excretion and CCCR than the PHPT patients.

We found no difference in plasma 25OHD between the two groups, but the PHPT patients had 36% higher average P- $1,25(OH)_2D$ levels than the FHH patients ($2p<0.01$). Adjustment for age, sex, BMI, season (summer/winter), and plasma levels of creatinine, phosphate and PTH did not change these findings.

Discussion

The present study demonstrates that average plasma 25OHD levels are normal, and average plasma $1,25(OH)_2D$ levels increased in the matched FHH group ($N=46$) compared with population based sex-, age-, and season-matched normal controls. Matching is

Table 2 Adjusted multiple regression analysis (partial correlation coefficients) of relationship between plasma levels of 25-hydroxyvitamin D (25OHD), 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), or parathyroid hormone (PTH) as the dependent variable and biochemical variables in patients with familial hypocalciuric hypercalcemia (FHH). Model: dependent variable, age, sex, BMI, and one other independent variable.

FHH	25OHD			1,25(OH) ₂ D		PTH	
	Adjusted for age, sex, and BMI			Adjusted for age, sex, and BMI		Adjusted for age, sex, and BMI	
	N	<i>r_p</i>	P	<i>r_p</i>	P	<i>r_p</i>	P
P-Calcium ionized (mmol/l)	66	-0.23	0.08	-0.11	0.40	0.34	<0.01
P-Calcium total (mmol/l)	66	-0.26	0.04	-0.13	0.31	0.30	0.02
P-Calcium alb. adj. (mmol/l)	66	-0.20	0.11	-0.08	0.54	0.39	<0.01
P-Creatinine (μmol/l)	66	0.23	0.07	-0.05	0.71	0.34	<0.01
P-PTH (pmol/l)	66	-0.06	0.67	0.20	0.12	-	-
P-Phosphate (mmol/l)	66	0.24	0.06	-0.06	0.63	-0.21	0.10
25OHD (nmol/l)	66	-	-	0.16	0.22	-0.06	0.67
1,25(OH) ₂ D (pmol/l)	66	0.16	0.22	-	-	0.20	0.12
Creatinine clearance (ml/min)	56	0.02	0.88	0.004	0.98	-0.47	<0.01
24H-U-Calcium (mmol)	56	0.18	0.20	0.18	0.20	0.11	0.43
Calcium/creatinine clearance ratio	56	0.16	0.25	0.20	0.16	0.23	0.10

Figure 1 Plasma PTH versus plasma 25OHD in familial hypocalciuric hypercalcaemia (FHH) and primary hyperparathyroidism (PHPT). Curve estimation model: power transformation.

a way to reduce bias caused by known confounders. The non-matched FHH patients were 11 men, 4 pre-, and 5 postmenopausal women. They did not differ significantly from the matched patients in age, height, weight, BMI, plasma PTH, phosphate, calcium measurements, season, or vitamin D metabolites, but they had significantly higher plasma creatinine levels ($2p < 0.01$) compared with the matched FHH patients.

With respect to 25OHD, our results are in accordance with a previous small Danish study performed between September 1982 and February 1983 (19). It included only ten FHH patients from one family and ten normal controls matched for age and sex and comparable

Figure 2 Seasonal variation in plasma 25OHD nmol/l based on single measurements in 66 patients with familial hypocalciuric hypercalcaemia (FHH; mean $\pm$ 95% CI).

regarding weight and body surface. Also, Law *et al.* found comparable 25OHD levels in 32 FHH patients and 65 normal controls (18, 22). Unfortunately, these data were not adjusted for season and BMI. In contrast to our study, Kristiansen *et al.* (19) were unable to detect any differences in 1,25(OH)₂D levels between FHH patients and normal controls, but this could be due to the small number of individuals investigated.

Figure 3 Scatterplot depicting the relationship between the *CASR* mutations and 1,25(OH)₂D (5).

Table 3 Comparison of descriptive and biochemical variables between patients with familial hypocalciuric hypercalcemia (FHH) and primary hyperparathyroidism (PHPT).

	FHH		PHPT		2p
	N	Mean (s.d.) or %	N	Mean (s.d.) or %	
Age (years)	66	51 (16.1)	147	60 (14.4)	<0.01
Females (%) ^a	66	66.7		78.9	0.06
Height (m) ^b	66	1.69 (0.09)	136	1.66 (0.09)	0.02
Body weight (kg) ^b	66	70.5 (15.1)	143	75.6 (17.0)	0.04
BMI (kg/m ²) ^b	66	24.5 (4.0)	135	27.3 (5.4)	<0.01
P-Calcium ionized (mmol/l) ^b	66	1.44 (0.10)	140	1.46 (0.12)	0.32
P-Calcium total (mmol/l) ^b	66	2.70 (0.15)	141	2.77 (0.21)	0.02
P-Calcium alb. adj. (mmol/l) ^b	66	2.75 (0.16)	147	2.80 (0.19)	0.04
P-Creatinine (μmol/l) ^b	66	73 (15.6)	147	79 (14.7)	<0.01
P-PTH (pmol/l) ^b	66	5.8 (2.65)	147	14.1 (12.23)	<0.01
P-Phosphate (mmol/l)	66	0.92 (0.19)	135	0.87 (0.18)	0.04
P-25OHD (nmol/l) ^b	66	64 (27.6)	147	63 (27.4)	0.69
P-1,25(OH) ₂ D (pmol/l) ^b	66	127 (40.5)	113	173 (64.6)	<0.01
Creatinine clearance (ml/min)	56	109 (28.3)	128	93 (33.8)	<0.01
24h-U-Calcium (mmol) ^b	56	3.3 (2.03)	129	8.2 (4.36)	<0.01
Calcium/creatinine clearance ratio ^b	56	0.008 (0.006)	117	0.23 (0.009)	<0.01

^aFisher's exact test.^bt-test based on logarithmic transformation of values.

The lack of reduction in plasma 25OHD in the hypercalcemic FHH patients compared with normal individuals support the view that either plasma Ca^{2+} *per se* does not affect dermal vitamin D production or the effect is neutralized by the inactivating mutation in the *CASR* gene i.e., the reduced sensitivity toward hypercalcemia. It probably also means that the effect of the increased average plasma 1,25(OH)₂D levels on the renal 24-hydroxylase is limited in these patients. However, the levels of 25OHD depend largely on individual life style and this may mask the possible effect of 1,25(OH)₂D on 24-hydroxylase activity. The borderline significant variation in 25OHD levels (one-way ANOVA, $P=0.06$) in the *CASR* mutations included in this study supports the possibility that plasma Ca^{2+} directly or indirectly may affect plasma 25OHD.

We have also disclosed that plasma 25OHD shows seasonal variations in FHH. This is in accordance with the findings in normal Danish individuals (33), in Danish lactating women (34), and in Danish patients with PHPT (6). In the latter study, plasma 25OHD covaried with monthly sun hours and satellite-based estimation of monthly UV exposure.

It is noteworthy that plasma levels of 25OHD in our study were unrelated to the renal 24-h calcium excretion and to the CCCR. It has previously been shown that in PHPT patients, the renal calcium excretion is positively related to plasma 25OHD even when adjusted by age, sex, BMI, creatinine, and season (6). The present finding could imply that vitamin D status and its effect on intestinal calcium absorption is of minor importance for the renal calcium excretion in FHH patients compared with the influence of the different *CASR* mutations. The lack of association between 25OHD, renal 24-h calcium excretion and CCCR in FHH also supports the idea that individual

vitamin D status is of limited importance for the use of CCCR as a diagnostic tool.

The increase in plasma 1,25(OH)₂D compared with controls was positively related to plasma PTH and inversely to plasma Ca^{2+} and plasma creatinine. This is in accordance with the stimulatory effects of PTH on the renal 1 α -hydroxylase and the inhibitory effects of hypercalcemia and reduced renal function (9, 35–38). The inverse correlation with plasma Ca^{2+} after adjustment for plasma PTH in the model suggests that plasma Ca^{2+} still may suppress the renal 1 α -hydroxylase in spite of the inactivating mutations in the *CASR*. We could not demonstrate any effect of phosphate on 1,25(OH)₂D concentrations in our FHH patients, although hypophosphatemia also is thought to enhance the 1 α -hydroxylase (9). Overall, changes in 1,25(OH)₂D levels were limited and mainly took place within the normal reference range. This is in accordance with our finding that plasma 1,25(OH)₂D did not depend on the different *CASR* mutations observed in the study.

In FHH, 23% of patients had elevated plasma PTH that correlated positively to plasma calcium and inversely to creatinine clearance. The usually observed inverse hyperbolic relationship between 25OHD and plasma PTH (33, 34, 39, 40) could not be retrieved in the present FHH population in contrast to the findings in the PHPT population. Other studies support that PTH varies inversely with vitamin D status in PHPT patients (6, 8). One explanation could be that the main cause of raised PTH levels in FHH is the inactivating mutation in the *CASR* and not secondary hyperparathyroidism provoked by vitamin D deficiency. This is supported by the significantly different effects of various *CASR* mutations on plasma PTH and plasma Ca^{2+} levels (5). Another explanation could be the lack of power due to the limited number of patients investigated.

Our comparison of FHH and PHPT patients disclosed that despite similar levels of plasma Ca^{2+} , the patients with PHPT had higher PTH levels, lower plasma phosphate, and higher $1,25(\text{OH})_2\text{D}$ in spite of slightly reduced renal function. The lack of difference in plasma 25OHD supports the study by Law *et al.* (18, 22), who found comparable 25OHD levels but significantly higher levels of $1,25(\text{OH})_2\text{D}$ in 11 PHPT patients compared with 32 FHH patients. Davies *et al.* (17) also found increased $1,25(\text{OH})_2\text{D}$ levels in 14 PHPT patients compared with 14 FHH patients after matching for serum calcium, creatinine clearance rate, and 25OHD. The higher $1,25(\text{OH})_2\text{D}$ levels in PHPT may be explained by the higher plasma PTH levels and the more reduced plasma phosphate levels in PHPT compared with FHH patients, since both variables stimulate the renal 1α -hydroxylase (9, 10).

In the clinical setting, the separation between FHH and PHPT is often based on the CCCR (20, 41, 42). We recommend a two-step diagnostic procedure in hypercalcemic patients with clinical suspicion of PHPT or FHH. The first step should be a CCCR determination with a cut-off value of <0.020 . This will exclude around two out of three of the PHPT patients and sample 98% FHH patients (20). The next step should be a CASR gene analysis in the sampled group, separating those with significant mutations (FHH) from those without (PHPT) (20).

Limitations to study

The strength of the present study is the uniform, routine diagnostic program applied in all cases. The most important weakness is probably the omission of CASR gene studies in the PHPT patients. However, all PHPT patients remained normocalcemic 2 months after surgery and none of them have been readmitted for recurrent hypercalcemia and most of them had adenoma, not hyperplasia as seen in some cases of FHH (43).

We used different vitamin D assays for patients and normal controls due to the time lag between sampling. However, we have tried to address this question by adjusting the measurements in the normal controls to be equivalent to the measurements in the two patient groups. The equations used for these corrections were based on double measurements in 123 patients for 25OHD and in 74 patients for $1,25(\text{OH})_2\text{D}$ (32). The large material used for correction makes it unlikely that the differences in laboratory methods will cause sizeable differences in measured values after correction.

Molecular genetic analysis is unable to detect large rearrangements in the CASR gene, and may therefore neglect some cases of FHH (5). Locus heterogeneity may also exist (44). It is at present unknown whether FHH could be a risk factor for later development of PHPT, so that the two diseases, in rare cases, may occur together (45, 46). In the present study, it is unlikely that some

participants had a combination of FHH and PHPT. First, all patients classified as having PHPT were normocalcemic 2 months after successful parathyroidectomy with a positive histological finding. Secondly, coexisting parathyroid adenoma was excluded by neck ultrasound and SPECT and planar parathyroid ($^{99\text{m}}\text{Tc}$ -Sestamibi) and thyroid scintigraphy in the mutation positive FHH index patients and by neck ultrasound in all hypercalcemic relatives. In PHPT patients, both ultrasound and parathyroid scintigraphy have previously been shown to have positive predictive values above 80% for correctly localized adenomas. When both techniques were positive, the positive predictive value rose to 97% (47). We consider imaging to be a safe and non-invasive way of evaluating the status of the parathyroid glands.

Conclusion

Compared with normal controls, FHH patients have elevated plasma $1,25(\text{OH})_2\text{D}$ and unchanged plasma 25OHD levels. However, patients with FHH had lower plasma levels of $1,25(\text{OH})_2\text{D}$ and PTH, but comparable plasma levels of Ca^{2+} and 25OHD compared with PHPT patients.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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