Should the *GCM2* gene be tested when screening for familial primary hyperparathyroidism?

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Abstract

Objective: Primary hyperparathyroidism (PHPT) is a disease with either sporadic or inherited presentation. Germline mutations responsible for this disease can be found in different genes, the most frequently involved being *MEN1*, *CDC73* = *HRPT2* and *CASR*. During the last few years, new genes have been described as responsible for the development of PHPT such as *GCM2*. These genes are not systematically included in PHPT genetic screening yet. The aim of this work was to assess the importance of *GCM2* genetic analysis in PHPT to determine if this gene should be included in gene panel investigated for this disease.

Design and methods: The TENGEn network (French Oncogenetic Network of Neuroendocrine Tumors) collected and interpreted allelic variants according to the clinical characteristics of the *GCM2*-positive patients identified through genetic testing performed in French laboratories (713 patients with PHPT).

Results: From 713 patients with PHPT included in this study, 85 (6.6%) carried at least one *GCM2* variant. A total of 12 variants classified as uncertain significance or likely pathogenic were reported in 47 patients. Their mean age at PHPT diagnosis was 49 years. Additionally, the investigation of a large family showed that *GCM2* variants could be associated with low penetrance.

Conclusion: We provide a description and interpretation for *GCM2* variants identified in a French population. We suggest that this gene should be included in genetic screening of patients with PHPT and propose the follow-up of asymptomatic patients carrying such variants for calcemia.

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Introduction

Primary hyperparathyroidism (PHPT) is a disorder characterized by hypercalcemia and elevated or inappropriate parathyroid hormone (PTH) secretion due to

parathyroid gland hyper-functioning. It can result from a single or multiglandular lesion, adenoma, hyperplasia or much less frequently carcinoma. The prevalence of this

disease is estimated around 1–7 cases per 1000 persons (1). PHPT can be either sporadic or inherited. Among inherited PHPT (5–10% of cases), some belong to syndromic diseases such as multiple endocrine neoplasia type 1 (MEN1; due to mutations in *MEN1* tumor suppressor gene), multiple endocrine neoplasia type 2A (MEN2A; due to mutations in *RET* proto-oncogene), multiple endocrine neoplasia type 4 (MEN4; due to mutations in *CDKN1B* proto-oncogene) or hyperparathyroidism-jaw tumor syndrome (HPT-JT; due to mutation in *CDC73=HRPT2*). PHPT can also be non-syndromic and isolated. The cases of familial isolated PHPT (FIHP) can be included in incomplete expression of MEN1, MEN4, HPT-JT or associated with mutation in *CASR*, *GNA11* or *AP2S1* genes (2, 3). In recent years, activating mutations in *GCM2*, a gene previously known to be involved in inherited hypoparathyroidism, have been described in PHPT (4, 5). Cases of PHPT associated with *GCM2* mutations in literature are characterized by multiple enlarged parathyroid glands, greater preoperative parathyroid hormone levels than patients without *GCM2* mutations, persistent or recurrent PHPT after surgery and higher risk of parathyroid carcinoma (6). Currently, frequency of *GCM2* mutation in FIHP is estimated to be around 18% in literature (5).

GCM2 is located in locus 6p34. It encodes for a 506 amino acid protein composed of several functional domains: a DNA-binding domain, 2 transactivation domains, a C-terminal conserved inhibitory domain (CCID) and a nuclear localization signal. This protein, which is exclusively expressed in parathyroid cells, acts as a transcription factor involved in parathyroid gland differentiation and development and in PTH production. Knockout mice for *GCM2* are characterized by parathyroid agenesis and hypoparathyroidism (7). *GCM2*-inactivating mutations have been involved in hypoparathyroidism and more recently, activating germline mutations in this gene have been described in association with hyperparathyroidism, suggesting that *GCM2* is a parathyroid proto-oncogene. These gain-of-function mutations are mostly located in the CCID domain (5).

Currently, genetic screening recommendation for PHPT in France do not include *GCM2* analysis. The aim of our study was to investigate germline *GCM2* sequence variations in a French cohort of patients with PHPT and to classify the identified variants according to ACMG-AMP guidelines (8, 9) in order to determine if this gene should be systematically included in PHPT gene screening decision tree. The deleterious effects of some sequence variants are controversial in literature and are still open for debate.

Material and methods

GCM2 sequencing

Patients selected in this study between 2015 and 2018 presented with a familial history of PHPT or exhibited a sporadic presentation before the age of 50 years, recurrent/persistent PHPT or multiple gland parathyroid lesions, according to French guidelines of genetic screening for PHPT (available at <http://www.reseau-gte.org/>). They all signed an informed consent allowing germline DNA analysis during a one-on-one genetic counseling. The study was validated and approved by Ethical Committee (Comité de Protection des Personnes (CPP Nord-Ouest IV)) under number HP 1930. In total, 713 independent probands were included in the study. Blood samples were addressed to one of the four French laboratories from the TENGEn network (French oncogenetic network on neuroendocrine tumors) analyzing *GCM2*.

DNA was extracted from leucocytes and sequenced by targeted next-generation sequencing. Sequence variations in *GCM2* exons and exon–intron boundaries were all confirmed by Sanger sequencing (primer sequences available on demand).

Additionally, 13 relatives from a family, presenting or not with primary hyperparathyroidism, were screened on two independent blood samples for a *GCM2*-specific variant by targeted Sanger sequencing. They also all signed an informed consent.

Classification of GCM2 variants

Variants are written according to HGVS nomenclature (with NM_004752.3, assembly GRCh37/hg19 version of human genome reference).

Sequence variants in *GCM2* were interpreted and classified according to ACMG-AMP guidelines taking into account open access population database frequencies (dbSNP, gnomAD, ESP, ExAc), *in silico* prediction softwares, the available familial, clinical and biological data and scientific publications. Classification for each variant in one out of the five classes was validated by the French TENGEn expert group (class 1: benign; class 2: likely benign; class 3: uncertain significance; class 4: likely pathogenic; class 5: pathogenic).

Results

Seven hundred thirteen patients with PHPT were included in this study. Excluding a very frequent variant identified

in about 10% of the DNA samples and directly considered as a benign class 1 variant (c.-44T>C, rs16870746, 10.73% in gnomAD(2.1)), 85 patients carried at least one GCM2 variant.

To be more accurate, we identified 12 variants classified as class 1 or 2 variants (benign or likely benign) according to ACMG criteria (Table 1). Interestingly, some of these variants were cosegregating variants such as c.679A>G, p.(Ile227Val) associated with c.350C>T, p.(Ala117Val) and c.462G>A, p.(Lys154Lys) associated with c.607G>A, p.(Gly203Ser). These class 1 or 2 variants were not taken into account for genotype–phenotype correlation, as they were very frequent and considered as SNP.

Twelve sequence variations were classified as class 3 (uncertain significance) or 4 (likely pathogenic) variants (Table 2). No sequence variation was classified in class 5 (pathogenic). Forty-seven index cases harbored these classes of variants, which correspond to a frequency of 6.6% (47/713). More accurately, 44 patients were carriers of a class 3 variant and three patients carried a class 4 variant. Interestingly, all these sequence variations were substitutions. We identified no deletion or duplication in these classes of variants. Main clinical characteristics of the corresponding patients are presented in Table 3. For these patients, mean age of PHPT diagnosis was 49 years (min: 13; max: 82). Nine patients harbored parathyroid hyperplasia, 17 patients presented with parathyroid adenoma and anatomopathological data were not available for the other patients.

In parallel of the study of this cohort, we had the opportunity to perform genetic investigations in a family (genealogic tree in Fig. 1). The index case, a 28-year-old man, presented with a 20 mm parathyroid adenoma associated with a calcemia level of 4.15 mmol/L and a

PTH level of 355 pg/mL. Our genetic analyses allowed to identify the c.1181A>C, p.(Tyr394Ser) class 4 variant. Interestingly, above the 13 relatives analyzed in this family, we identified this variant in 7 of them: four members carrying this variant at heterozygous state but without PHPT respectively at the age of 80 (Ca: 2.4 mmol/L and PTH: 30.2 pg/mL), 58 (Ca: 2.3 mmol/L and PTH: 6 pg/mL), 34 and 33. The three other members of this family harboring the variant had a symptomatic PHPT.

Discussion

In our study, 47 patients out of 713 were carriers of a class 3 or class 4 variant, none of them carried a class 5 variant. This represents 6.6% of the patients screened for PHPT in our population. Guan *et al.* (5), who selected kindreds with FIHP, found a frequency of 18% of patients with a GCM2 variant and without deleterious mutation on the current other genes involved in heritable hyperparathyroidism. This observed discrepancy can be due to selection of familial cases in their study and to prior exclusion of mutation in *MEN1*, *CASR*, and *CDC73*, while our cohort included all patients screened for heritable PHPT, including sporadic cases. Sporadic presentation in case of genetically determined PHPT is not so rare because PHPT can be asymptomatic over many years, knowledge of first- but especially second- or third-degree relative affected with PHPT is not always obvious, and incomplete penetrance of pathology with genetic abnormality is also to be considered. That is the reason why genetic screening is recommended in France for all patients of less than 50 years with PHPT, even with sporadic presentation. The mean age of PHPT diagnosis in patients carriers of a class 3 or 4 GCM2 variant in our

Table 1 Classes 1 and 2 variants. Frequencies in African population and results of functional evaluations are indicated when available and informative for interpretation.

Nucleotide variation	Protein variation	Frequency in gnomAD (v2.1) (All; %)	Results of functional studies
c.-44T>C	p.(?)	10.73	
c.344-7T>C	p.(?)	0.13	
c.350C>T	p.(Ala117Val)	0.11 (AFR: 1.2%)	
c.462G>A	p.(Lys154Lys)	1.0 (AFR: 10.28%)	
c.607G>A	p.(Gly203Ser)	1.0 (AFR: 10.26%)	NS (5)
c.679A>G	p.(Ile227Val)	0.25 (AFR: 2.7%)	NS (5)
c.739C>T	p.(Leu247Leu)	0.002	
c.897T>C	p.(Pro299Pro)	0.87 (AFR: 5.06%)	
c.903T>C	p.(Asp301Asp)	0.86	
c.1060A>G	p.(Met354Val)	0.41 (AFR: 4.16%)	
c.1177_1185dup	p.(Ala393_Gln395dup)	0.13	
c.1395C>T	p.(His465His)	0.027	

NS, no significant effect on transcription in comparison with wild-type sequence.

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Table 2 Classes 3 and 4 variants.

Nucleotide variation	Protein variation	ACMG class proposition	Number of patient harboring this variant	Frequency in gnomAD (All: %)	Results of functional studies
c.-13G>A	p.(?)	3	1	0.0044	
c.35T>A	p.(Val12Glu)	3	1	0.0016	
c.139C>T	p.(Arg47Cys)	3	1	0.002	
c.157G>A	p.(Asp53Asn)	3	2	0.086	
c.168G>C	p.(Lys56Asn)	3	2	0.077	
c.319G>A	p.(Asp107Asn)	3	1	0.053	
c.809G>A	p.(Arg270Lys)	3	1	NA	
c.844T>G	p.(Tyr282Asp)	3	30	0.9	Controversial effects (4, 5, 14)
c.943A>G	p.(Asn315Asp)	3	5	0.34	Activating effect (5)
c.989A>T	p.(Gln330Leu)	3	2	0.028	
c.1181A>C	p.(Tyr394Ser)	4	3	0.062	Activating effect (5)
c.1217G>A	p.(Arg406Gln)	3	1	0.023	

study was 49 years, which stands between mean age of parathyroidectomy of family presenting PHPT patients (42.8 years) and sporadic presenting PHPT patients (59.2 years) in the study of El Lakis *et al.* (1).

Castellano *et al.* (10) reported a female-to-male ratio of 3:1 in PHPT patients. In our study, this ratio was 2:1 in patient carriers of a class 3 or 4 *GCM2* variant. This more equilibrated female-to-male ratio is consistent with a genetic predisposition to the pathology and could thus be explained by the Mendelian transmission.

El Lakis *et al.* (1) also demonstrated that activating mutations of *GCM2* were associated with a greater rate of multi-glandular diseases. In our study, some patients with class 3 or 4 variants harbored parathyroid hyperplasia or multiple glandular adenomas but the lack of anatomopathological information available for many patients did not allow us to confirm this point.

A great number of variants are still classified as class 3 variants (variant of uncertain significance). Indeed, even when *in vitro* functional data are available, their interpretation can remain tricky, as for the c.844T>G, p.(Tyr282Asp) variant. In our cohort, this variant has been identified in association with a deleterious variant once in *MEN1* gene and once in *CASR* gene. As a transcription factor expressed in parathyroid cells and activating PTH transcription, *GCM2* can contribute to development of PHPT but, may be, to a lesser extent than *MEN1*, *CDKN1B* or *HRPT2* (11). Interaction with *CASR* expression in parathyroid cells has been described, as well as interaction with *GATA3* and *MafB* for *PTH* expression, but some compensation mechanisms seem to exist (12, 13). This could explain the low penetrance observed, as shown with our family study.

The pathogenicity of p.(Tyr282Asp) variant is controversial in literature and its role in tumorigenesis

is not proven (14). D'Agruma *et al.* (4) demonstrated that this variant was associated with hypercalcemia and exhibited increased transcriptional activity compared to the wild-type protein. In the same way, Marchiori *et al.* (15) showed that this variant was associated with increased serum Ca levels. On the contrary, Guan *et al.* (5) demonstrated that the transcriptional activity of this variant was similar to the wild-type sequence. This variant is not localized in the CCID domain where activating mutations have mostly been identified. The mutated position is not well conserved among species and *in silico* predictions mainly predict the variant as benign (six prediction software considered it as benign vs three pathogenic). Frequency of p.(Tyr282Asp) is currently of 1.23% in ESP database (European American). This frequency over 1% in population could tilt the balance in favor of the interpretation of this variant as a benign polymorphism, even if the threshold of 5% is not reached, which would constitute a stand-alone evidence of benign impact according to ACMG-AMP guidelines. Anyway, an enrichment of this sequence variation is noticed in patients with PHPT, as initially described by D'Agruma *et al.* (4). It is also the case in our cohort in comparison with the control population of gnomAD v2.1 database (30/1426 VS. 2549/282896, OR 2.3 (IC95: 1.6 to 3.4)), but too moderately to be considered as an argument for a pathogenic effect. Moreover, in our cohort, this variant has been identified in two patients with pathogenic variation in another gene: one patient had a pathogenic inactivating substitution in *CASR* (NM_000388.3 c.164C>T) and the other patient had a deletion in *MEN1* (NM_130799.2 c.776del). Finally, the selected criteria for ACMG classification could be as follows: PS4 (The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in

Table 3 Characteristics of patients with a class 3 or 4 variant.

Patient	Sex	Year of birth	Age at diagnosis	c.DNA sequence variation	Protein sequence variation	ACMG classification	Clinical and biological presentation	Familial history	Parathyroid anatomopathological data	Other variation in PHT gene panel
1	M	1973	44	c.139C>T	p.(Arg47Cys)+p.(Gln330Leu)	3	Isolated PHPT	No	NA	No
2	F	1960	56	c.-13G>A	p(?)	3	Isolated PHPT Ca: 2.78 mmol/L PTH: 254 pg/mL	No	Hyperplasia	No
3	M	1998	13	c.168G>C+ c.157G>A	p.(Lys56Asn)+p.(Asp53Asn)	3	Isolated PHPT Ca: 2.83 mmol/L PTH: 136 pg/mL	No	Adenoma	No
4	M	1951	65	c.168G>C+ c.157G>A	p.(Lys56Asn)+p.(Asp53Asn)	3	Hypercalcemia Ca: 2.60 mmol/L PTH: 70 pg/mL	No	NA	No
5	F	1946	70	c.319G>A	p.(Asp107Asn)	3	Isolated PHPT Ca: 2.70 mmol/L PTH: 196 pg/mL	No	NA	No
6	M	1986	24	c.35T>A	p.(Val12Glu)	3	Isolated PHPT Ca: 3.38 mmol/L PTH: 222 pg/mL	Yes (sister: renal colic)	adenoma	No
7	F	1971	47	c.809G>A	p.(Arg270Lys)	3	Isolated PHPT	NA	NA	No
8	F	1935	82	c.844T>G	p.(Tyr282Asp)	3	Isolated PHPT	Yes (daughter: hypercalcemia)	Multiple adenoma	No
9	F	1942	75	c.844T>G	p.(Tyr282Asp)	3	Hypercalcemia Ca: 2.75 mmol/L	NA	NA	No
10	F	1988	29	c.844T>G	p.(Tyr282Asp)	3	Isolated PHPT	No	NA	No
11	F	1948	66	c.844T>G	p.(Tyr282Asp)	3	Isolated PHPT	NA	Hyperplasia and multiple adenoma	No
12	F	1945	72	c.844T>G	p.(Tyr282Asp)	3	Isolated PHPT Ca: 2.53 mmol/L PTH: 195 pg/mL	No	Hyperplasia	No
13	F	1962	56	c.844T>G	p.(Tyr282Asp)	3	Isolated PHPT	No	Adenoma	No
14	F	1965	51	c.844T>G	p.(Tyr282Asp)	3	Isolated PHPT Ca: 2.73 mmol/L PTH: 120 pg/mL	NA	Hyperplasia	No
15	F	1986	31	c.844T>G	p.(Tyr282Asp)	3	Isolated PHPT	NA	Adenoma	No
16	F	1967	48	c.844T>G	p.(Tyr282Asp)	3	HPT and pancreatic neuroendocrine tumor	NA	NA	No
17	M	1965	50	c.844T>G	p.(Tyr282Asp)	3	Isolated PHPT Ca: 3.03 mmol/L PTH: 183 pg/mL	NA	Adenoma	No
18	M	1969	45	c.844T>G	p.(Tyr282Asp)	3	Isolated PHPT	No	Hyperplasia	No
19	F	1943	73	c.844T>G	p.(Tyr282Asp)	3	Isolated PHPT	No	Multiple adenoma	No
20	M	1969	44	c.844T>G	p.(Tyr282Asp)	3	Isolated PHPT	Yes (grandfather: renal lithiasis)	Adenoma	No
21	F	1976	39	c.844T>G	p.(Tyr282Asp)	3	Isolated PHPT	No	Adenoma	No
22	F	1941	61	c.844T>G	p.(Tyr282Asp)	3	PHPT and pituitary adenoma	Yes (brother: renal colic)	Adenoma	No
23	F	1945	NA	c.844T>G	p.(Tyr282Asp)	3	Hypercalcemia and Cushing disease Ca: 2.61 mmol/L	No	NA	No
24	F	1981	30	c.844T>G	p.(Tyr282Asp)	3	Isolated PHPT	Yes (mother: renal lithiasis)	adenoma	No
25	F	1941	76	c.844T>G	p.(Tyr282Asp)	3	PHPT and gastrinoma	No	NA	No

(Continued)

Table 3 Continued.

Patient	Sex	Year of birth	Age at diagnosis	c.DNA sequence variation	Protein sequence variation	ACMG classification	Clinical and biological presentation	Familial history	Parathyroid anatomopathological data	Other variation in PHPT gene panel
26	M	1980	34	c.844T>G	p.(Tyr282Asp)	3	Isolated PHPT	No	Adenoma	No
27	F	1945	61	c.844T>G	p.(Tyr282Asp)	3	Isolated recurrent PHPT	NA	Adenoma	No
28	F	1950	65	c.844T>G	p.(Tyr282Asp)	3	Ca: 2.76 mmol/L Isolated recurrent PHPT	NA	NA	Yes (CASR ex2: c.164C>T)
29	F	1981	31	c.844T>G	p.(Tyr282Asp)	3	PHPT and pituitary adenoma Ca: 2.83 mmol/L PTH: 283 pg/mL Isolated PHPT	NA	NA	Yes (MEN1 ex4: c.776del)
30	F	1960	58	c.844T>G	p.(Tyr282Asp)	3	Ca: 2.71 mmol/L PTH: 162 pg/mL Isolated recurrent PHPT	NA	Hyperplasia	No
31	M	1948	69	c.844T>G	p.(Tyr282Asp)	3	Ca: 2.93 mmol/L PTH: 751 pg/mL Isolated PHPT	No	Adenoma	No
32	F	1974	42	c.844T>G	p.(Tyr282Asp)	3	Isolated PHPT	Yes (mother: renal lithiasis)	NA	No
33	M	1995	23	c.844T>G	p.(Tyr282Asp)	3	Isolated PHPT	NA	Hyperplasia	No
34	F	1955	45	c.844T>G	p.Tyr282Asp	3	Hypercalcemia Ca: 2.71 mmol/L	NA	Hyperplasia	No
35	F	1968	NA	c.844T>G	p.(Tyr282Asp)	3	Hypercalcemia Ca: 2.63 mmol/L	No	Hyperplasia	No
36	M	1964	54	c.844T>G	p.(Tyr282Asp)	3	Isolated PHPT	No	Hyperplasia	No
37	F	1953	NA	c.844T>G	p.(Tyr282Asp)	3	Hypercalcemia	NA	Hyperplasia	No
38	F	1985	32	c.943A>G	p.(Asn315Asp)	3	Isolated PHPT	No	Hyperplasia	No
39	F	1972	45	c.943A>G	p.(Asn315Asp)	3	Isolated PHPT Ca: 3.04 mmol/L PTH: 203 pg/mL	No	Hyperplasia	No
40	M	1950	67	c.943A>G	p.(Asn315Asp)	3	Isolated PHPT Ca: 4.31 mmol/L PTH: 1111 pg/mL	No	Hyperplasia	No
41	M	1997	17	c.943A>G	p.(Asn315Asp)	3	Isolated PHPT Ca: 2.90 mmol/L PTH: 88 pg/mL	No	NA	No
42	M	1937	79	c.943A>G	p.(Asn315Asp)	3	Isolated PHPT Ca: 3.30 mmol/L PTH: 300 pg/mL	Yes (father: renal lithiasis)	2 adenoma	No
43	M	1984	33	c.989A>T	p.(Gln330Leu)	3	Isolated PHPT	Yes (2 maternal aunts and 2 paternal uncles: renal lithiasis)	1 adenoma	No
44	M	1988	28	c.1181A>C	p.(Tyr394Ser)	4	Isolated recurrent PHPT Ca: 4.15 mmol/L PTH: 355 pg/mL	Yes (father: renal lithiasis)	Adenoma	No
45	F	1945	71	c.1181A>C	p.(Tyr394Ser)	4	Isolated recurrent PHPT	No	Hyperplasia	No
46	M	1972	46	c.1181A>C	p.(Tyr394Ser)	4	Isolated PHPT	NA	NA	No
47	M	1985	30	c.1217G>A	p.(Arg406Gln)	3	Isolated PHPT	No	NA	No

hypercalcemia: hypercalcemia without significant increase of PTH; Isolated PHPT, hypercalcemia and increase of PTH; NA, not available.

Figure 1

Genealogic tree of the family with *GCM2* (NM_004752.3) c.1181A>C, p.(Tyr394Ser) variant.

controls)+BP4 (Multiple lines of computational evidence suggest no impact on gene or gene product (conservation, evolutionary, splicing impact, etc...)) resulting in classification as a class 3 variant, that is variant of uncertain significance. PP5 criteria (Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation) was not applied because the functional effect of this variant is controversial in literature. All these elements could suggest that this sequence variation is a phenotype modifier variant, as it has been described for the frequent variant p.(Ala986Ser) in *CASR*, described as contributor to the pathogenesis but not as being responsible for the development of the pathology. Probably more experience and family studies will allow to classify more clearly this variant.

Among the 44 patients harboring a class 3 variant, excepting p.(Tyr282Asp) variant, 14 patients harbored another variant. This represents about 2% of our cohort which is not negligible. This highlights the necessity for carrying on the investigations on this gene.

Unlike p.(Tyr282Asp), the pathogenicity of the variant p.(Tyr394Ser), localized in the CCID domain, is well admitted in literature (14). It has been shown that this variant is enriched in Ashkenazi Jewish kindreds with PHPT (16). It was identified in three index cases in our cohort. The ages of onset were 28, 46 and 78 years.

The youngest was a male who presented with severe hypercalcemia linked to unique adenoma and family history of PHPT, the second one was a female with recurrent parathyroid hyperplasia and the third one was a male with PHPT, but no more clinical information was available. Thus, heterogeneity of clinical and anatomopathological presentations are observed with this variant. Incomplete penetrance associated with this *GCM2* sequence variation was also highlighted by the family study presented in this article, with two siblings of more than 55 years old presenting with normal calcium and phosphate levels. Our observation is in agreement with the recent article of Riccardi *et al.* (17) who studied *GCM2* variants localized in CCID in sporadic PHPT and concluded that the penetrance of these variants was low. We thus classified this variant as a class 4 variant (likely pathogenic).

Notwithstanding the limits of our study due to lack of some biological and anatomopathological data, we suggest that *GCM2* sequencing should be included in the systematic genetic screening of PHPT for patients with a familial history of PHPT or exhibiting a sporadic presentation before the age of 50 years or with recurrent/persistent PHPT or with multiple gland parathyroid lesions. Next-generation sequencing facilitates this process (18). However, the interpretation of the sequence variations identified in *GCM2* is quite difficult, due to the lack of functional studies available in routine diagnosis

laboratory to determine the consequences of the variants, and the incomplete penetrance of likely pathogenic variants. This also highlights the importance of discussion between geneticists and clinicians in multidisciplinary concentration meetings and validation of variants by expert groups such as TENGEn in France, which achieves it for many years for *MEN1* variants (19). So, in front of incomplete penetrance and variants of uncertain significance, what about management of patients harboring those variants? Follow-up of asymptomatic patients harboring such a variant is not excessively binding. They should just be monitored for calcemia and PTH to be able to detect the onset of the pathology, which can happen very late in life indeed never as the penetrance is incomplete.

To conclude, we propose to include this gene in panels for PHPT screening. The proposition of genetic screening for all patients of less than 50 years with PHPT appears to be still valid, as diagnosis of genetically determined PHPT may be younger for patients with *HRPT2* or *MEN1* mutation but older for patients with *CASR* or *GCM2* mutation. Up till now, many *GCM2* variants are classified as variants of uncertain significance. However, improvement in the future of our knowledge about this gene will help us in the tricky interpretation and classification of such variants.

Declaration of interest

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

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