Diagnosis of Asymptomatic Primary Hyperparathyroidism: Proceedings of the Third International Workshop

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Objective: Asymptomatic primary hyperparathyroidism (PHPT) is a common clinical problem. The purpose of this report is to guide the use of diagnostic tests for this condition in clinical practice.

Participants: Interested professional societies selected a representative for the consensus committee and provided funding for a one-day meeting. A subgroup of this committee set the program and developed key questions for review. Consensus was established at a closed meeting that followed. The conclusions were then circulated to the participating professional societies.

Evidence: Each question was addressed by a relevant literature search (on PubMed), and the data were presented for discussion at the group meeting.

Consensus Process: Consensus was achieved by a group meeting. Statements were prepared by all authors, with comments relating to accuracy from the diagnosis subgroup and by representatives from the participating professional societies.

Conclusions: We conclude that: 1) reference ranges should be established for serum PTH in vitamin D-replete healthy individuals; 2) second- and third-generation PTH assays are both helpful in the diagnosis of PHPT; 3) DNA sequence testing can be useful in familial hyperparathyroidism or hypercalcemia; 4) normocalcemic PHPT is a variant of the more common presentation of PHPT with hypercalcemia; 5) serum 25-hydroxyvitamin D levels should be measured and, if vitamin D insufficiency is present, it should be treated as part of any management course; and 6) the estimated glomerular filtration rate should be used to determine the level of kidney function in PHPT: an estimated glomerular filtration rate of less than 60 ml/min · 1.73 m^2 should be a benchmark for decisions about surgery in established asymptomatic PHPT. **(*J Clin Endocrinol Metab* 94: 340–350, 2009)**

The Third International Workshop on Asymptomatic Primary Hyperparathyroidism (PHPT) was convened with the support of 10 international sponsoring societies to review the advances made in its diagnosis and management since the last workshop which was held in 2002 (1). The PHPT Task Force, constituted by representatives from all the sponsoring societies, was charged with developing questions pertaining to diagnosis, medical management, surgical management, and preoperative imaging. Four separate working groups considered these questions. This report focuses on the diagnosis of PHPT and provides the first of the four separate reports presenting the consensus of the task force.

The key questions addressed were:

Question 1. Do we now have optimal reference intervals for serum PTH? Are these intervals based on individuals who are vitamin D replete?

Question 2. Do third-generation PTH assays perform better clinically than second-generation PTH assays for the diagnosis of PHPT?

Question 3. Have sequence tests for the calcium-sensing receptor (*CASR*) gene, multiple endocrine neoplasia (MEN)-related genes, and other genes become suitable for routine evaluation of some forms of PHPT?

Question 4. Is normocalcemic hyperparathyroidism a part of the diagnostic spectrum of PHPT?

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Abbreviations: CASR, Calcium-sensing receptor; CT, computed tomography; FHH, familial hypocalciuric hypercalcemia; FIH, familial isolated hyperparathyroidism; GFR, glomerular filtration rate; h, human; MEN, multiple endocrine neoplasia; 25OHD, 25-hydroxyvitamin D; PHPT, primary hyperparathyroidism.

Question 5. Should we measure serum 25-hydroxyvitamin D (25OHD) in all patients with suspected PHPT? How should the different reference ranges for different assays be interpreted? What represents the threshold for overtreatment?

Question 6. Which imaging study is appropriate for detection of renal stones: plain abdominal radiographs, ultrasound, or computed tomography (CT)? Do we have accurate reference intervals to determine what is at least 30% below the glomerular filtration rate (GFR) expected for age?

An electronic literature search was conducted using PubMed on all published literature between 1996 and June 2008. Keywords were combined to identify the relevant articles. Studies were classified according to the study design (Table 1). Manuscripts focused on the key questions were prepared by the team members. These manuscripts were presented at the Workshop on May 13, 2008 in Orlando, Florida. After the presentations and discussions, a consensus panel convened, and each question was discussed in detail. Through discussion and dialogue, consensus was achieved. The recommendations presented in this document reflect the opinion of the panel.

Question 1. Do we now have optimal reference intervals for serum PTH? Are these intervals based on individuals who are vitamin D replete?

Although the major synthetic product of the parathyroid cell is an 84-amino acid peptide, considerable intraglandular and extraglandular metabolism of this molecular species occurs, resulting in a heterogeneous assortment of circulating fragments (1). This was initially realized when first-generation PTH assays were developed in the 1960s and 1970s (1). These assays used multivalent antibodies raised against parathyroid extracts of various species, PTH (1–84) preparations of varying degrees of purity or synthetic peptides as standards, and purified ^{125}I-PTH (1–84) or region-specific ^{125}I-synthetic fragments as tracers (1). The main epitopes that these assays recognized were either in the carboxyl (C) terminal domain (region 53–84) or the mid C-terminal domain (region 44–68). About 20% of the immunoreactivity detected by these assays was chromatographically similar or identical to PTH (1–84), whereas the remaining 80% was comprised of smaller C-PTH fragments (1). Comparison between assays was difficult because of the different relative units used to express PTH results and because of the different ranges employed by each assay. The capacity of these assays to distinguish nonparathyroid hypercalcemia from mild PHPT was limited because production of C-PTH fragments is stimulated by chronic hypercalcemia, and they will be detected in both hyperparathyroidism and nonparathyroid hypercalcemia (2). Furthermore, these fragments are cleared by the kidney and accumulate in renal failure, further complicating interpretation of results (2).

Because of the difficulties these assays presented, including the fact that they measured mainly fragments, they were replaced by a second-generation of PTH assays more specific for intact PTH (1–84) (1). This second-generation of PTH assays first became available in 1987 in the form of the Nichols Institute Allegro Intact PTH Assay (3). This two-site immunoradiometric assay employed two immunoaffinity purified antibodies, a capture antibody purified against human (h) PTH (39–84) and a revealing antibody purified against hPTH (1–34). The main epitope recognized by this latter antibody was in the 13–34 region of hPTH (1–34) (1). This assay revolutionized PTH measurement because it was easy to use, employed a purified hPTH (1–84) standard, and permitted direct quantitative comparisons between studies. Furthermore, it improved the discrimination between normal individuals and patients with PHPT or nonparathyroid hypercalcemia (3). It was also more useful to assess patients with renal failure who had increased PTH secretion (3); the PTH guidelines of the National Kidney Foundation Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines for patients with chronic kidney disease were developed with this assay (4). It had a reference interval of 12 to 65 ng/liter and was initially determined in 72 sera of normocalcemic individuals (3). The Nichols PTH assay no longer exists and has been replaced by numerous similar second-generation assays, which, however, produce PTH values varying between 50 and 150% of the expected Nichols PTH values (5, 6). This has been problematic in the application of Q/DOQI PTH guidelines in chronic kidney disease because the guidelines employed absolute PTH values that were developed with the Nichols assay (4). It has been less problematic in the diagnosis of PHPT because the upper ranges of these assays, irrespective of their absolute values, are used to define abnormalities consistent with PHPT.

The second-generation PTH assays were subsequently demonstrated to react with relatively long fragments of hPTH (1–84) present in the circulation (7), which started their amino acid structure at positions 4, 7, 10, and 15 of hPTH (1–84), positions 7 and 15 being predominant. These fragments represent 20% of the PTH immunoreactivity in normal individuals but up to 50% in renal failure patients because PTH fragments accumulate in renal failure (7). They have been reported to oppose the classical biological effects of hPTH (1–84) on serum calcium (8, 9), on urinary phosphate excretion (8), and on 1,25 dihydroxyvitamin D synthesis (10), but the physiological relevance of these observations remains to be determined. To eliminate these large C-

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TABLE 1. Evidence for recommendations

Hierarchy of evidence	Refs.
Level Ia: Systematic review of randomized controlled trials	
Level Ib: Single randomized controlled trials	26, 36, 45, 55, 71
Level II: Systematic review of observational studies	17, 35
Level III: Single observational studies, low-quality randomized controlled trials	1–3, 6–15, 19, 21, 24, 25, 27, 28, 33, 37–46, 48–50, 52, 56, 58–60, 61–70, 72–75, 78–83
Level IV: Case series, surveys	5, 16, 18, 20, 22, 23, 30–32, 34, 51, 54, 58, 68
Level V: Consensus guidelines	4, 53, 77

PTH fragments that include a partially absent amino (N) terminal region, a third-generation of PTH assays was designed.

The third-generation of PTH assays was introduced in 1999 (11), and the first of these, the Whole PTH Assay from Scantibodies Laboratory Inc., used a capture antibody raised against hPTH (39–84) and a revealing antibody raised against a small N-terminal peptide, with an epitope in region 1–4 (11). As expected, these assays provided results that were about 20% lower in normal individuals and up to 50% lower in renal failure patients than did second-generation assays. The reference interval for the third-generation Whole PTH Assay is 7 to 36 ng/liter, defined in 135 normocalcemic individuals (11). Third-generation assays were eventually found to recognize a second molecular form of PTH (1–84), immunologically intact at both extremes, which reacted only poorly in second-generation PTH assays and which had 13–24 epitopes, related to a suspected posttranslational phosphorylation on serine 17 (12). This molecular species represented less than 10% of the immunoreactivity in normal individuals and up to 15% in renal failure patients. It has been, however, overexpressed in a limited number of patients with a severe form of primary adenomatous hyperparathyroidism or with parathyroid cancer (13). Paradoxically, therefore, in these specimens, third-generation assays provide higher PTH results than second-generation assays.

In addition to the problem of molecular heterogeneity, PTH concentrations have been reported to be influenced by a number of other factors that may impinge upon establishing a reference interval (14); thus, for example, PTH elevations have been described in older individuals (15–18), especially older women (19); in blacks relative to whites (20); in those with low calcium intake (21–23); and in obese individuals (24, 25). Establishment of PTH reference ranges, however, did initially not take into account these other factors. In most cases, the PTH elevations in these situations have been attributed to secondary hyperparathyroidism, with vitamin D deficiency or insufficiency as a common factor driving elevated PTH.

Certainly, elevations of PTH have consistently been found to occur in vitamin D deficiency and insufficiency. However, an international consensus on a reference range for 25OHD levels, the best available index of vitamin D nutritional status, has not yet been achieved. A standard definition of an optimal 25OHD level has been the concentration above which PTH cannot be suppressed further, or the concentration below which PTH levels begin to rise. Estimates of optimal 25OHD concentrations reached using the PTH suppression criterion vary widely [from 30 to 110 nmol/liter (8–44 ng/ml) (26–31)], although the largest study in a healthy population would suggest an optimal level of 80 nmol/liter (30 ng/ml) (30). Part of this uncertainty may reside with standardization and operator variability in 25OHD assays (32) because a variety of methods to measure 25OHD are available and different extraction procedures are used. The International Vitamin D External Quality Assessment Scheme (DEQAS) is an effort to harmonize 25OHD assays among different laboratories (32) and may be an important step in the right direction, which finally might facilitate the development of a standard and universally agreed-upon reference range for 25OHD.

Attempts to identify a reference range for 25OHD have also examined other endpoints, besides the PTH level. Calcium absorption is one index. Although serum 25OHD concentrations have been related to calcium absorption and these studies indicated that calcium absorption at 50 nmol/liter (20ng/ml) was significantly lower than at a mean 25OHD level of 86 nmol/liter (34 ng/ml), they did not establish a 25OHD level at which optimal calcium absorption would occur (33). It is much more difficult to use bone mineral density (33) and reductions in fractures (35, 36) to establish a threshold. Another approach to establishing a threshold is to evaluate the impact of vitamin D supplementation on PTH levels, and such an approach supported a lower threshold of 50 nmol/liter (or 20 μg/liter) (28).

Souberbielle *et al.* (37), using a second-generation PTH assay, pointed out how important it is to exclude subjects with low serum 25OHD levels in establishing a reference range for PTH. When they excluded such subjects, the upper limit of the PTH reference interval for second-generation assays decreased from 65 to 46 ng/liter, a 29% reduction. In addition, when vitamin D-deficient individuals were excluded from the subjects used to establish a reference interval for "Whole PTH," the upper limit also decreased from 44 to 34 ng/liter, a 27% reduction; the upper limit of the reference interval thus remained lower for third-generation assays than for second-generation ones. Nevertheless, in their studies, values below 50 nmol/liter (20 ng/ml) were taken as the lower limit of vitamin D sufficiency, whereas other studies have reported that PTH levels in normocalcemic individuals continue to decline until levels of 25OHD above 75nmol/liter (30 ng/ml) have been achieved (18, 36, 38, 39). This illustrates the importance of establishing an international consensus on a reference range for 25OHD, if we are to improve the reference range of PTH by excluding subjects with vitamin D insufficiency.

Conclusions

We do not have optimal reference intervals for PTH values based on coexisting 25OHD levels. Further studies are required to establish reference intervals for second- and third-generation PTH assays using large population cohorts that are comprised of vitamin D-replete subjects and also to stratify according to age, sex, race, GFR, and possibly body mass index.

Question 2. Do third-generation PTH assays perform better clinically than second-generation PTH assays for the diagnosis of PHPT?

Third-generation PTH assays detect N-PTH, a posttranslationally modified form of PTH (1–84) in region 15–20 as well as PTH (1–84). Because N-PTH normally represents less than 10% of third-generation PTH results in normal individuals and up to 15% in end-stage renal disease, it can be estimated that N-PTH accounts for less than 10% of the difference between second- and third-generation results overall. Exceptions to this rule have been described so far in about 10 patients who overexpressed N-PTH (12, 13, 43), some in the setting of a severe form of PHPT or parathyroid cancer (12, 13). In all cases, this has been paradoxically detected by the finding of higher third-generation than second-generation PTH assay results (12, 13, 43).

Only four studies have so far compared the sensitivity of second-generation *vs.* third-generation PTH assays in the diagnosis of PHPT (41, 44, 46). They are summarized in Table 2, which has been modified from a recent publication (47). These studies summarize 405 hypercalcemic patients with the disease, 341 proven surgically (47). Females constituted more than 80% of the patients in the three studies where gender information is given (44–46), mean age was near 61 yr, and mean total calcium concentration was near 11 mg/dl in the same studies (44–46). The upper limit of the PTH normal range used to define elevated PTH values differed from one study to another. It was the upper normal range defined by the kit supplier in two studies (41, 44), or an upper range defined in 70 normocalcemic postmenopausal women in another (45), or a vitamin D-sufficient range defined in 74 normal subjects in the last study (46). The second-generation, Nichols Allegro Intact PTH assay identified 195 of 221 subjects with elevated PTH values adding the results of two studies (41, 44), a sensitivity of 88.2%.

Similar values were obtained for the Scantibodies Total PTH assay of 168 of 184 or 91.3% adding two studies (45, 46) and 141 of 145 or 97.3% with the Nichols Advantage Intact assay in a single study (46). For third-generation assays, the Scantibodies Whole PTH assay identified 351 of 405 elevated PTH values when results of the four studies were combined (41, 44–46), resulting in a sensitivity of 89.1%, whereas the Nichols Advantage Bio-Intact assay detected 129 of 145 or 89% in a single study (46). If the four studies are combined (41, 44–46) for second- and third-generation PTH assays, taking the mean of the two second-generation and the two third-generation PTH assays in the Boudou *et al.* study (46), detection rates are 363 of 405 or 89.6% for second-generation PTH assays and 365 of 405 or

TABLE 2. Sensitivity of second- and third-generation PTH assays for the diagnosis of PHPT (modified from Ref. 47)

First author, year (Ref.)	Demographic and biochemical data						PTH assay sensitivity			
							Second-generation		Third-generation	
	No. of patients	Sex (M/W)	Age (yr)	Samples	Mean Ca_t (mg/dl)	Control group	Nichol's allegro intact PTH	Scantibodies laboratory total PTH	Nichol's advantage intact PTH	Scantibodies laboratory whole PTH	Nichol's advantage bio-Intact PTH
Gao, 2001 (41)	165	?	?	EDTA plasma	?	No	151/165 (91.5%)			155/165 (93.9%)	
Reference range						Assays range	10–65 pg/ml			7–36 pg/ml	
Silverberg, 2003 (44)	56	7/49	60 ± 2	Serum	10.9 ± 0.1	No	41/56 (73%)			54/56 (93%)	
Reference range						Assays range	10.65 pg/ml			5–31 pg/ml	
Carnevale, 2004 (45)	39	0/39	63.9 ± 8.2	Serum	11.0 ± 0.88	Yes		32/39 (82%)		30/39 (77%)	
Reference range						70 postmenopausal women		10–65 pg/ml		8–44 pg/ml	
Boudou, 2005 (46)	145	28/117	61.7 ± 3.2	Serum	11.2 (9.52–11.24)	Yes		136/145 (93.8%)	141/145 (97.3)	122/145 (84.2%)	129/145 (89%)
Reference range						74 subjects D >50 nmol/liter		10–46 pg/ml	11–60 pg/ml	8.4–34 pg/ml	9–41 pg/ml
All	405	30/205	Mean >60		Mean near 11	Variable	195/221 (88.2%)	168/184 (91.3%)	141/145 (97.3%)	351/405 (89.1%)	129/145 (89%)

Results are means ± SD or range. ?, Unknown; Ca_t, total serum calcium.

90.1% for third-generation PTH assays, which are essentially identical results (41, 44–46). All Nichols commercial PTH assays were removed from the market in 2006. With the still available Whole and Total PTH assays from Scantibodies, sensitivity is at 89.1 and 91.3%, respectively, even with the use of a different reference range normalized for the female population studied or the vitamin D status (45, 46).

These results indicate that the sensitivity of second- and third-generation PTH assays in detecting elevated PTH values is similar. It remains to be demonstrated whether these results will differ when reference ranges are established using large population cohorts that are restricted to vitamin D-replete subjects, and stratified according to age, sex, race, and possibly body mass index.

Conclusions

There is no overall difference between second- and third-generation assays for the diagnostic evaluation of PHPT; however, both of these newer generation assays represent an improvement over the first-generation PTH assay.

Question 3. Have sequence tests for the *CASR* gene, MEN-related genes, and other genes become suitable for routine evaluation of some forms of PHPT?

The value of genetic testing in familial hyperparathyroidism and other genetic forms of PHPT is considered in this section. Emphasis is placed upon the genes associated with familial hypocalciuric hypercalcemia (FHH), MEN type 1 (MEN1), MEN2, and the hyperparathyroidism jaw-tumor syndrome. The value of gene sequence analysis in the assessment of the four main hyperparathyroid syndromes has been addressed after identification of the syndromal genes (*RET* in 1993, *CASR* in 1993, *MEN1* in 1997, and *HRPT2* in 2003). Gene sequencing is available from both academic and commercial laboratories.

The evidence about genetic testing

Genetic tests for carriers of a mutation and/or carriers of a syndrome

For the present discussion, genetic tests are directed at identifying the carrier state for a genetic hyperparathyroid disorder. They are not recommended for use in direct evaluation of DNA in a parathyroid tumor because such testing is not useful for syndrome diagnosis or for tumor staging. A possible exception could be tumor *HRPT2* mutation testing for diagnosis of parathyroid carcinoma, but this requires further study. Traditionally, genetic tests were based on highly penetrant traits. Examples in MEN1 would include serum calcium or, as more recently recognized, skin angiofibromas. Assessment of such traits still has an important place, particularly when DNA testing is not a good option. Gene sequencing for mutation detection has become the gold standard (when positive in a proband) because of its feasibility at any age and the high penetrance of the detected mutations (approaching 100% in familial MEN2A).

Distinct roles for gene sequence tests

Genetic tests, and particularly gene sequence tests have several different and related roles.

- First, a syndrome may be confirmed in a proband. Confirming a syndrome provides information regarding possible pathological processes that may develop, such as increased incidence of parathyroid carcinoma in hyperparathyroidism-jaw tumor syndrome. The pretest level of suspicion of a syndrome may be variable. For example, families with atypical FHH may rarely be identified by DNA analysis, leading to avoidance of inappropriate parathyroid surgery.
- Second, testing in the proband can determine whether a shortcut or exon-specific test can be offered to relatives in that family. Because all carriers in one syndromal family share an identical mutation, only a small zone of sequence about that mutation needs to be amplified for testing.
- Third, and in parallel with the above, testing in asymptomatic relatives can establish or refute a member as a silent carrier. In the latter situation, a negative test will spare the subject forever from the need for ongoing biochemical and other screening. When a mutation cannot be identified in an affected family member, the same gene can sometimes be screened by linkage or haplotype testing with nearby polymorphisms. Assessment of the so-called asymptomatic relative might theoretically include the possibility of prenatal or preimplantation testing, which has rarely been done in a hyperparathyroid syndrome.
- Fourth, trait testing in a known carrier should be done periodically to screen for emergence of potentially morbid syndromal tumors in parathyroid and other tissues.
- Fifth, a positive test may lead to a major intervention. An example of this is the presence of an activating mutation in the *RET* gene in asymptomatic relatives, with consideration given to early thyroidectomy to prevent or cure medullary thyroid carcinoma. At the present time, preventive parathyroidectomy is not recommended for any of the familial hyperparathyroid syndromes, with the possible exception of hyperparathyroidism-jaw tumor syndrome for the prevention of the possible parathyroid carcinoma.

FHH

Familial traits

FHH has traditionally been diagnosed in families by the presence of hypercalcemia and relative hypocalciuria. Hypercalcemia is present in virtually all carriers including neonates (48, 49). The degree of hypercalcemia seen in FHH is similar to that in individuals with PHPT. It is recommended that all family members be evaluated once the diagnosis is established. Hypercalcemia is seen in children under the age of 10 in FHH, a finding that is almost never present in other forms of familial hyperparathyroidism. The calcium to creatinine clearance ratio is of particular value and is usually below 0.01 in FHH, and this ratio is usually above 0.01 in typical PHPT. There is a modest overlap in these values. It is important to ensure that other causes of hypercalcemia and relative hypocalciuria are excluded, including concurrent treatment with thiazide diuretics or lithium.

Traits in an isolated case

The approach to a first case, or an isolated case, or a proband is different from the assessment of kindred. Features suggestive of FHH are hypercalcemia and relative hypocalciuria, hypercalcemia with normal PTH in the normal reference range, and persistent hypercalcemia after an attempted parathyroidectomy. Definitive diagnosis requires genetic studies of the *CASR* gene.

The CASR gene sequence testing

The genetic basis in the majority of FHH families is mutation of the *CASR* gene, judging by their linkage to chromosome 3q (50). Rare families with the same syndrome are caused by mutation of one of two unidentified genes mapped by linkage to chromosome 19p and 19q. However, about 30% of the families linked to 3q do not have an identifiable *CASR* mutation. Presumably, there are *CASR* mutations (for example the promoter region is not tested routinely) invisible to current methods. Expense, limited need, and the high false-negative rate limit the use of *CASR* testing. Testing serum and urine calcium in three relatives has a lower false-negative rate in family diagnosis than doing a DNA sequence in a proband. However, a genetic test for a *CASR* mutation in a case of unclear hypercalcemia can avoid unnecessary surgery.

CASR gene testing is of greater value in the following circumstances: 1) hypocalciuric hypercalcemia in an isolated patient without access to additional family members for evaluation; and 2) familial isolated hyperparathyroidism (FIH) in the absence of classical features of FHH (51). Approximately 10% of the FIH kindreds are due to *CASR* gene mutations (52). *CASR* gene sequence testing in FHH families is not required on an urgent basis. Its purpose is essentially to confirm the diagnosis and ensure that inappropriate parathyroid surgery is avoided. Family members being evaluated for FHH should have serum calcium tested, preferably before the age of 20.

MEN1

Defining MEN1

In MEN1 there is a predisposition to tumors of the parathyroid, pancreatic, and pituitary glands (53). Familial MEN1 requires MEN1 to be present in the presence of one or more first-degree relatives with at least one of the three tumors. This definition does not imply that all MEN1 cases are from the same gene. For example, the prevalence of identifiable *MEN1* mutation is 70% in familial MEN1 but only 7% in sporadic MEN1 with both parathyroid and pituitary tumor (54). We will focus upon the hyperparathyroidism of MEN1. Hyperparathyroidism is the most frequent endocrine expression of MEN1, reaching 95% by age 50. Gastrinoma is less frequent but potentially carries a greater morbidity due to the presence of excessive gastric acid production and the possibility of metastatic disease.

The hyperparathyroid trait in MEN1

This trait in MEN1 is expressed somewhat similarly to that in sporadic parathyroid adenoma. Important differences are an average onset age (20 yr) that is 30 yr younger than in adenoma, a 1:1 gender ratio, parathyroid tumor multiplicity, a high rate of recurrence (50% at 10 yr) after an apparently successful subtotal parathyroidectomy, and specific tumor(s) outside the parathyroid in the patient or a relative. Onset has been noted as early as age 8, but kidney stones and advancing hypercalcemia have not been noted before age 12. The earlier onset is associated with osteopenia at an earlier age than in adenoma.

Trait testing to diagnose a carrier of MEN1

Before gene identification in 1997, trait testing was the sole method for diagnosis (excluding rarely used linkage or haplotype testing about chromosome 11q13). The traits that have been most useful for diagnosis are ionized calcium, PTH, and prolactin. Skin tumors such as facial angiofibroma and truncal collagenoma are common and very specific for MEN1, however their usefulness in screening has not been established.

MEN1 gene sequence testing

Since 1997, *MEN1* sequence testing has been offered in academic and commercial labs. Its roles are as listed above. Among the 30% of typical probands without identified *MEN1* mutation, one other gene, *p27*, has so far been implicated in two MEN1 probands (55, 56). Named as MEN4 by OMIM, this syndrome is a rare cause and may account for about 1% of all MEN1 probands. Like the *CASR* test in FHH, the *MEN1* test in MEN1 is not urgent. The main role is to give long-term information to patients, relatives, and care providers.

Trait testing for emergence of hyperparathyroidism in a known carrier

Hyperparathyroidism in euparathyroid carriers of MEN1 should be screened for with calcium and PTH annually. Periodic screening for nonparathyroid tumors in known carriers is not covered further here.

MEN2A

MEN2A is another autosomal dominant disorder. The mutation in the MEN2A gene (*RET*) is identifiable in 95% of familial MEN2A (53). The typical tumors are medullary thyroid carcinoma (in 95% cases), pheochromocytoma (in 50%), and parathyroid (in 30%). There is some genotype/phenotype correlation; mutations in codon 634 are commonly associated with the additional hyperparathyroidism (53), and so annual measurement of serum calcium is recommended. MEN2A rarely presents as FIH. DNA analysis for mutations in the *RET* oncogene is of value in considering prophylactic thyroidectomy to prevent the development of medullary thyroid carcinoma. Silent parathyroid tumors in MEN2A may also be discovered during such a procedure.

Hyperparathyroidism-jaw tumor syndrome

This is an autosomal dominant disorder. Parathyroid tumors are the most common manifestation, often with the asynchronous development of multiple adenomas (49, 57). Parathyroid carcinoma develops with greatly increased frequency (15–20%). Other features include ossifying fibromas of the mandible and maxilla, and renal lesions such as cysts and hamartomas. Mutation of the *HRPT2* (*CDC73*) gene is detectable in about 70%

of cases, and as a tumor suppressor, most of its mutations are easily seen to predict inactivation of the gene product parafibromin (57). Heritable *HRPT2* mutations are also found in a subset of patients with sporadic presentation of parathyroid carcinoma (58) and in a minor proportion of kindreds with FIH (59). *HRPT2* DNA testing in relatives can result in the identification and surveillance of individuals at risk for malignant parathyroid disease, raising new questions about treatments and enabling preventative or curative treatment, and this should be seriously considered in these contexts.

FIH

FIH is a broad category used for the familial syndromes that do not fit into any of the categories above (excluding neonatal severe PHPT that we do not cover due to its rarity). It has no specific features, and some cases must represent occult presentation of an above-mentioned syndrome (49, 52). Most cases have unknown causes, probably unrelated to the four main genes (above) (52). Testing of *CASR*, *MEN1*, and *HRPT2* can be recommended because it has implications for the proband and the family, but the total yield of detecting any mutation is low (<20%), and the expense is substantial. Family features that might make a syndromal mutation more likely include multigland parathyroid disease, parathyroid cancer, and onset of hypercalcemia before age 20.

Conclusions

DNA sequence testing for mutations of *CASR*, *MEN1*, and *HRPT2* genes can provide clinically useful information, particularly in known or suspected cases of familial hyperparathyroidism. These studies are not recommended on a routine basis. Mutations in the *RET* gene are of particular value in the management of medullary thyroid carcinoma in MEN2A.

Question 4. Is normocalcemic hyperparathyroidism a part of the diagnostic spectrum of PHPT?

This condition is being increasingly identified with the availability and more widespread usage of PTH assays. This condition is indeed a part of the diagnostic spectrum and is addressed in more detail in the article by Silverberg *et al.*, in this issue of the *journal*. In making the diagnosis, it is critical to exclude other causes of elevated PTH and normal serum calcium (Table 3).

TABLE 3. Common causes of secondary hyperparathyroidism (modified from Ref. 85)

Disorder	Comment
Chronic kidney disease (CKD)	When GFR decreases below 60 ml/min (stage 3 or greater CKD)
Drugs	Bisphosphonates, anticonvulsants, furosamide, phosphorus
Hypercalciuria due to renal leak	Renal hypercalciuria
Malabsorption syndrome	Celiac disease, cystic fibrosis
Vitamin D insufficiency	Plasma 25-OHD < 50 nmol/liter (20 μg/liter)
Pseudohypoparathyroidism type 1b	Such cases can be normocalcemic

Question 5: Should we measure 25-OHD in all patients with suspected PHPT? How should the different reference ranges for different assays be interpreted? What represents the threshold for overtreatment?

The prevalence of vitamin D inadequacy is common, approaching epidemic proportions, both in general population and in patients with PHPT in particular. Despite this rising prevalence of vitamin D depletion, there is a pervasive, but unfounded, practice of restricting both calcium and vitamin D supplements in PHPT for fear of aggravating hypercalcemia, which in most patients is stable over extended periods of time (60). Vitamin D inadequacy has been implicated in several aspects of PHPT, such as parathyroid tumorigenesis, adenoma weight, severity of the disease, and in the pathogenesis of osteitis fibrosa cystica (61, 62). In addition, lower serum levels of 25-OHD are associated with higher serum PTH levels, lower bone mineral density at the hip and forearm, higher bone turnover markers, such as alkaline phosphatase (63), and somewhat paradoxically higher serum calcium levels compared with patients without vitamin D depletion (64). Finally, vitamin D inadequacy has been associated with a higher risk of developing postoperative hypocalcemia, hungry bone syndrome (62), and persistent postoperative elevated serum PTH levels (64).

Measurement of serum 25-OHD is currently the best available test to assess vitamin D adequacy (61) and thus should be measured in all patients with PHPT. The serum levels of 25-OHD are often reduced in patients with PHPT (61–65) and may mask hypercalcemia in some patients mimicking normocalcemic PHPT (for details, refer to the article by Silverberg *et al.*, in this issue of the *journal*). Indeed, a standard high dose vitamin D therapy (the so-called vitamin D challenge test) to uncover such "normocalcemic" patients was in vogue before the availability of PTH assays; however, with the availability of improved PTH assays this test is no longer needed or recommended (see question 1 for details).

There are a few studies on the prevalence of vitamin D depletion in patients with PHPT (61–65). In a series from Denmark, vitamin D insufficiency [defined as serum 25-OHD levels <50 nmol/liter (<20 ng/ml)] was found in 81% of patients with PHPT compared with 60% in controls (63). In a series of studies from the United States, a similar high prevalence of vitamin D depletion (50%) was found with a cutoff level of 50 nmol/liter (20 ng/ml) (61, 64, 65). In all studies, the prevalence of vitamin D depletion was much higher than in the controls (63) or in patients with osteoporosis (66, 67). The mechanism for the low serum 25-OHD levels is believed to be due to an accelerated catabolism of 25-OHD in patients with PHPT; it is speculated that this is mediated by the increased serum levels of PTH and 1,25-dihydroxyvitamin D (68).

There is increasing evidence that vitamin D deficiency may actually worsen the clinical picture in PHPT (61–65). Early case reports of severe vitamin D deficiency associated with PHPT have been noted (69, 70) in which correction of the severe deficiency results in a rise in serum calcium. This observation has perhaps caused physicians to be cautious in replacing vitamin D in patients with PHPT. Although prudence is always to be recommended, vitamin D replacement has not resulted in further increases in serum calcium. It has, however, been associated with reductions in serum PTH. In a recent randomized clinical trial of surgical *vs.* medical management of PHPT, routine supplementation of vitamin D 400 IU/d was not associated with either higher serum calcium or urine calcium excretion (71). In the only systematic study of pharmacological administration of high-dose vitamin D, Grey *et al.* (72) administered vitamin D_3 50,000 IU (1.25 mg) weekly for 4 wk, followed by once a month for 1 yr to 21 women with PHPT and coexisting vitamin D insufficiency [defined as 25-OHD levels <50 nmol/liter (20 ng/ml)]. After 1 yr, there was no change in serum calcium, but serum PTH levels decreased by 26%, and serum alkaline phosphatase levels decreased significantly. The peak serum 25-OHD levels were between 55 and 115 nmol/liter, with a mean of 77 nmol/liter after 1 yr of supplementation. Although further studies are needed to confirm these observations, it is reasonable to assume that either routine (71) or even pharmacological vitamin D supplementation (72) is not associated, in most patients, with aggravation of existing hypercalcemia or increased urine calcium excretion (71, 72).

Due to a significant prevalence of vitamin D insufficiency in individuals with PHPT and the benefits seen with vitamin D supplementation in this population, it is recommended that 25-OHD levels be measured in all patients with PHPT. Identification of vitamin D inadequacy is necessary for appropriate management. The relationship between serum 25-OHD and PTH has been evaluated, and PTH plateaus in some studies when serum 25-OHD levels reach 50 nmol/liter (28). It would be appropriate to consider vitamin D supplementation in all individuals with PHPT if serum 25-OHD levels are below 50 nmol/liter. Evidence to support this is, however, limited to the study by Grey *et al.* (72). Further studies are required to determine the optimum level of 25-OHD in patients with PHPT. The "threshold value" for 25-OHD adequacy could be different in PHPT compared with those who do not have PHPT. Nevertheless, it is our recommendation that measurement of serum 25-OHD levels be performed in all patients with PHPT and that correction of vitamin D depletion is warranted before other management decisions (medical or surgical).

Notwithstanding the obvious theoretical, clinical, and practical implications of vitamin D depletion in patients with PHPT, precise measurement of serum levels of 25-OHD remains a challenge because the assay variability is high and the calibration between assays is poor. Not unexpectedly, the intra- and inter-assay variability is much greater at lower levels compared with higher levels of serum 25-OHD (73). Although, the mean serum 25-OHD level differs depending on the assay method used (RIA, chemiluminescence, or liquid chromatography-tandem mass spectrometry), fortunately the relative ranking is similar between assays (73). The DEQAS is an effort to harmonize 25-OHD assays among different laboratories (32, 74) and may be an important step in standardizing assays that will facilitate development of a reference range for 25-OHD. Nevertheless, these assay problems should not influence routine measurement of serum 25-OHD or correction of vitamin D depletion because the latitude between "reference" and "toxic" levels is quite wide. It would appear from the study of Grey *et al.* (72) that a serum level of 25-OHD up to115 nmol/liter is safe in PHPT and that a much higher level is required before any adverse events occur. A recent review indicated that, apart from a single extremely unusual case, there have been no reports of vitamin D toxicity with serum 25-OHD levels below 280 ng/ml (or 700 nmol/liter) (75).

Conclusions

Vitamin D deficiency is common in patients with PHPT, and measurement of serum 25-OHD levels is recommended routinely. Vitamin D deficiency should be treated before making any medical or surgical management decisions. It is recommended that serum 25-OHD be maintained above 50 nmol/liter.

Standardization of the clinical laboratory measurement of serum 25-OHD assays is needed. It is also recommended that further research be conducted to determine the optimal vitamin D levels for individuals with PHPT, including randomized clinical trial data with vitamin D supplementation.

Question 6: Which imaging study is appropriate for detection of renal stones: plain abdominal radiographs, ultrasound, or CT? Do we have accurate reference intervals to determine what is at least 30% below GFR expected for age?

In PHPT the presence of undiagnosed calcium stones and or nephrocalcinosis categorizes the patient as having symptomatic disease, and despite the absence of renal symptoms in such patients they should not be described as asymptomatic. Renal calcium stones and nephrocalcinosis can be detected by several imaging techniques. In the context of asymptomatic PHPT, these include a plain radiograph of the abdomen, ultrasound scan of the kidneys, and CT of the abdomen. Previous recommendations for the identification of kidney stones has been to obtain a plain radiograph of the abdomen and pelvis or to obtain an ultrasound. Plain radiography is readily available but has serious limitations because of overlying gas and tissues. Ultrasound has many advantages including sensitivity, low cost, availability, and absence of radiation and has become very popular (78, 79). Spiral CT of the abdomen has advantages in sensitivity and anatomical location of the calcium deposits, although the instrument is not as widely available as ultrasound. Nonenhanced spiral CT in a prospective comparison with ultrasound had a higher sensitivity for the detection of ureteral stones in patients with renal colic.

Conclusions (Part 1)

It is recommended that renal imaging be performed if kidney stones are suspected. Ultrasound is the recommended imaging

modality of choice, followed by CT scan if addition imaging is required.

The National Kidney Foundation Kidney Disease Outcomes Quality Initiative (KDOQI) does not recommend the use of 24-h urinary collections for creatinine to estimate creatinine clearance (4). It recommends the estimation of GFR from equations based on anthropomorphic criteria (age, gender, race, weight) and serum measurements (creatinine, albumin, urea, nitrogen). In adults, equation no. 7 derived from the Modification of Diet in Renal Disease study (MDRD equation no. 7) (81) and the Cockcroft-Gault equation (82, 83) are both recommended. Exceptions to this recommendation do exist in individuals with exceptional dietary intake (vegetarian diet, creatine supplements) or markedly reduced muscle mass (amputation, malnutrition, muscle wasting).

Creatinine production is influenced by age, gender, and race (84). Men have higher creatinine levels than women, and among men non-Hispanic Black individuals have the highest creatinine levels (84), followed by non-Hispanic Whites and Mexican-Americans. Equations provide an estimate of GFR that takes these factors into account. The Cockcroft-Gault equation (82, 83) takes into account age (years), weight (kilograms), gender, and serum creatinine (milligrams per deciliter):

$$C_{cr}\ (\text{ml/min}) = \frac{[(140 - \text{age}) \times \text{weight}] \times (0.85 \text{ if female})}{(72 \times S_{cr})}$$

whereas the MDRD equation no. 7 (81) takes into account age (years), gender, race, serum creatinine (milligrams per deciliter), albumin (milligrams per deciliter), and urea nitrogen (milligrams per deciliter):

$$\text{GFR } (\text{ml/min} \cdot 1.73\ \text{m}^2) = 170 \times (S_{cr})^{-0.999} \times (\text{SUN})^{-0.170} \times$$

$$(\text{Alb.})^{+0.318} \times (\text{Age})^{-0.176} \times (0.762 \text{ if female}) \times$$

$$(1.180 \text{ if black})$$

GFR obtained with these equations or with 24-h urinary collections and adjusted for body surface (1.73 m^2) have been compared with the renal clearance of ^{125}I-iothalamate using data from the MDRD study (81). GFR estimated from 24-h urinary collection or by the Cockcroft-Gault equation tend to overestimate GFR relative to the MDRD equation no. 7 (81). Thus, we recommend the use of the MDRD equation because this is a more accurate estimate of GFR than the Cockcroft-Gault equation.

The KDOQI reported that there are no age-related reference internals of estimation of GFR based on subjects who are free of hypertension. It recommends using stages of chronic kidney disease; stage 3 corresponds with a GFR of 60 ml/min · 1.73 m^2 and would be a suitable threshold to adopt for concern about further renal compromise. This is also a cutpoint below which PTH levels begin to rise in individuals with chronic kidney disease in the absence of PHPT.

Conclusion (Part 2)

We recommend the use of a GFR of 60 ml/min · 1.73 m^2 as the threshold of chronic kidney disease for making decisions about surgery in patients with PHPT. Thus, we recommend the use of the MDRD equation because it is more accurate for estimating GFR than the Cockcroft-Gault equation.

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This paper summarizes the responses to key questions about the diagnosis of asymptomatic PHPT based on the Third International Workshop on Primary Hyperparathyroidism.

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