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Probability of Positive Genetic Testing Results in Patients with Family History of Primary Hyperparathyroidism

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Abstract

Background—Approximately 10% of patients with primary hyperparathyroidism (PHPT) have hereditary disease. Hereditary PHPT may be syndromic (multiple endocrine neoplasia (MEN1, 2, 4), hyperparathyroidism-jaw tumor syndrome) or non-syndromic (familial isolated primary hyperparathyroidism). There are limited data on the probability of testing positive for genetic mutation based on clinical presentation. The aim of this study was to determine potential associations between clinical and biochemical features and mutation in susceptibility genes for PHPT in patients with a family history of PHPT.

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Study Design—A retrospective analysis of 657 patients who had an initial parathyroidectomy for PHPT at a tertiary referral center. Logistic regression analyses were performed in 205 patients with a family history of PHPT to identify factors associated with a positive genetic test.

Results—Of 657 patients, 205 (31.2%) had a family history of PHPT. Of those 205, 123 (60%) had a germline mutation detected (91 *MEN1*, 14 *CDC73*, 18 *GCM2*). In univariate analysis, younger age (< 45 years old), male sex, multigland disease, and parathyroid carcinoma were associated with positive germline mutation; biochemical cure after an initial parathyroidectomy was less frequent in patients with familial PHPT (96.2% vs. 89.2%, $P = 0.005$). In multivariable analysis, age < 45, male sex, and multigland disease were independent factors associated with positive genetic testing.

Conclusions—In addition to a family history of PHPT, male sex, age < 45 years, and the presence of multigland disease should prompt physicians to offer the opportunity for genetic counseling and testing as it could influence the management of patients with PHPT.

Introduction

Primary hyperparathyroidism (PHPT) is a common disorder with an estimated prevalence of one to seven cases per 1,000 persons [1–3]. The incidence of PHPT is highest in the seventh decade of life, and it is more common in postmenopausal women, in whom the prevalence is as high as 3.2% [4]. PHPT may be sporadic or inherited in an autosomal-dominant pattern. Inherited PHPT may be part of a syndrome such as multiple endocrine neoplasia types 1, 2, and 4 (MEN1, 2, 4); hyperparathyroidism-jaw tumor syndrome (HPT-JT); or non-syndromic familial PHPT, referred to as familial isolated hyperparathyroidism (FIHP) [5–11].

Approximately 10% of PHPT is hereditary [5]. MEN1 occurs because of a germline loss-of-function mutation in the *MEN1* tumor suppressor gene. MEN1 is the most common familial form of PHPT, affecting 2–4% of PHPT patients. Almost all MEN1 patients will develop PHPT by age 50 and they are more likely to have multigland disease [11]. MEN2A occurs due to a gain-of-function germline mutation in the *RET* proto-oncogene. The penetrance of PHPT in MEN2A is approximately 35% [9]. HPT-JT is rare, and PHPT penetrance is variable. It is due to a germline inactivating mutation in the *CDC73* tumor suppressor gene and is associated with an increased risk of persistent/recurrent disease, as well as a higher frequency—up to 37.5%—of parathyroid carcinoma [8]. FIHP is an autosomal-dominant disorder characterized by the absence of a non-parathyroid clinical manifestation of known syndromic PHPT [6]. While all the susceptibility genes for FIHP have not been identified, approximately 18% are due to gain-of-function mutations in the *GCM2* proto-oncogene. GCM2-associated FIHP is commonly caused by multiple enlarged parathyroid glands and is associated with a higher risk of parathyroid cancer and persistent/recurrent PHPT after parathyroidectomy [6].

The distinction between sporadic and hereditary PHPT is important for several reasons. First, syndromic PHPT is associated with a risk of having or developing tumors at other sites that affect patient prognosis. Second, the initial surgical management of inherited PHPT may require a more comprehensive exploration due to a higher rate of multiple enlarged parathyroid glands, supernumerary parathyroid glands specifically in thymic tissue in

patients with MEN1 syndrome or parathyroid carcinoma. Third, the long-term follow-up of inherited PHPT requires lifelong surveillance for recurrent PHPT. Lastly, at-risk family members require screening and genetic counseling.

Several medical and surgical societies have established guidelines for genetic screening and counseling in the context of PHPT. For example, the American Association of Endocrine Surgeons parathyroidectomy guidelines recommend genetic counseling/testing in patients with PHPT who are younger than 40 years old and who have multigland disease, and in patients with either a suggestive family history or syndromic clinical manifestations [12]. The European Society of Endocrine Surgeons recommends *MEN1* genetic testing in PHPT patients who are younger than 40 years old with multigland disease or persistent/recurrent PHPT [13]. However, there are limited data on the probability of having a positive genetic testing result based on clinical presentation, biochemical profile and pathologic factors in a patient with a family history of PHPT.

In this study, we evaluated whether specific clinical or biochemical features or parathyroid pathology in patients with a family history of PHPT are associated with positive genetic testing results in four susceptibility genes (*MEN1*, *RET*, *CDC73*, *GCM2*).

Methods and Patients

Patients were enrolled into clinical protocols after providing written informed consent. These protocols were approved by the Institutional Review Boards of the National Institute of Diabetes and Digestive and Kidney Diseases and the National Cancer Institute (NCT01005654, NCT00001277). Patients with no family history of PHPT based on a family history questionnaire were defined to have sporadic PHPT (n = 452). All patients with inherited PHPT were diagnosed with MEN1 (n = 91) based on positive genetic testing for germline mutations in *MEN1*, with HPT-JT (n = 14) based on positive genetic testing for germline mutations in *CDC73*, and with GCM2-associated FIHP (n = 18) based on germline mutations in *GCM2*. Patients with PHPT were classified as having FIHP (n = 100) if they had at least one first-degree relative with PHPT in the absence of clinical or radiological evidence of syndromic disease or if they had germline mutations in *MEN1*, *RET*, *CDC73*, *GCM2*, and *CASR* (to exclude familial hypocalciuric hypercalcemia (FHH)) [6]. All MEN2A patients with PHPT were excluded as they presented with medullary thyroid carcinoma as their first manifestation and underwent neck surgery. Genetic testing for *MEN1*, *RET*, *CDC73*, *GCM2*, and *CASR* was performed in a Clinical Laboratory Improvement Amendments–certified laboratory with genetic counseling provided to patients and family members.

Baseline demographic and biochemical characteristics, including age, sex, ethnicity, serum calcium, intact parathyroid hormone (PTH), and type and extent of initial operative intervention and postoperative data, were collected and analyzed. Biochemical cure was defined as the presence of documented normal serum calcium levels for at least six months of postoperative follow-up time. Persistent disease was defined as elevated serum calcium and PTH within six months from the parathyroidectomy. Recurrent PHPT was defined as elevated serum calcium occurring six months or later after parathyroidectomy. Multigland

parathyroid disease was defined by the presence of two or more enlarged parathyroid glands. The age cutoff to discriminate between young and old patients with PHPT was selected based on Receiver Operator Characteristic (ROC) curve analysis (Area Under the Curve (AUC): 0.769; $P < 0.001$).

Statistical Analysis

Demographics, clinical and biochemical features, parathyroid pathology, and patient outcomes were compared in patients with and without a family history of PHPT using Student's t-tests, chi-square tests, and Mann-Whitney U tests as appropriate. A multivariable logistic regression model was derived that included age, sex, ethnicity, preoperative laboratory values and postoperative pathology. To obtain a simple and practical model, continuous variables such as age were dichotomized. Variables independently associated ($P < 0.05$) with the presence of germline mutations in the analysis were then ranked by the magnitude of the odds ratios. We then assessed the goodness of fit using Hosmer-Lemeshow statistics. All P values reported are two-tailed, and a P value < 0.05 was considered statistically significant. All statistical analyses were conducted using SPSS Statistics version 21.0 for Windows (SPSS, Inc., Chicago, Illinois).

Results

The clinical and biochemical characteristics compared between patients with and without a family history of PHPT are summarized in Table 1. Two hundred and five patients had a family history of PHPT in at least one first-degree relative: 91 MEN1, 14 HPT-JT, and 100 FIHP. Compared to the sporadic group, patients with familial PHPT were younger (mean age = 42.8 years vs. 59.2 years) and had more men (39% vs. 21%, $P < 0.001$), respectively. There was no significant difference in race/ethnicity, preoperative PTH, and serum total calcium. Patients with a positive family history had a higher rate of multigland disease (70.2% vs. 14.4%; $P < 0.001$) and parathyroid carcinoma (2.4% vs. 0%; $P = 0.003$) compared to the sporadic group. Based on at least two years of follow-up, patients in the sporadic group had a lower rate of persistent/recurrence PHPT than the familial group (3.8% vs. 10.8%; $P = 0.005$).

Male gender (Odds ratio (OR): 1.68; 95% confidence interval (CI): 1.02–2.77; $P = 0.041$), multigland disease (OR: 14.1; 95% CI: 8.9–22.5; $P < 0.01$), and age ≥ 45 (OR: 7.74; 95% CI: 4.7–12.8; $P < 0.01$) were independent predictors of having a positive germline mutation on multivariate analysis (Table 2). The probability of germline mutation in a PHPT susceptibility gene for a patient with positive family history was highest in male patients ≥ 45 years of age with multigland disease (92%) (Figure 1). The lowest rate of genetic mutation in a PHPT susceptibility gene was in females > 45 years of age with single gland disease (6%).

Discussion

Hereditary PHPT accounts for approximately 10% of all newly diagnosed cases of PHPT in which a germline mutation in *MEN1*, *CDC73*, *RET*, or *GCM2* has been identified [10, 14]. Routine genetic testing for patients with a family history of PHPT is not well established,

and clinical criteria are lacking to help identify which patients have the highest likelihood of testing positive [12]. In addition, given that PHPT is common in the general population, two first-degree relatives may have PHPT simply as a chance occurrence. Therefore, determining initial clinical, biochemical and pathology factors that may be associated with a higher probability of positive testing for a PHPT susceptibility gene is important in developing a cost-effective recommendation for additional testing, for counseling patients, and even when considering management and follow-up strategies.

In the current study and consistent with previous studies, we found that patients with a family history of PHPT presented at a younger age, had a higher frequency of multigland disease, parathyroid carcinoma and persistent/recurrent disease. Most importantly, we found that in our patients with a family history of PHPT, age ≥ 45 years at diagnosis and male gender were preoperative independent factors strongly associated with positive germline mutation; furthermore, we established that the presence of multigland parathyroid disease was an independent variable associated with positive germline mutations in these patients.

The rationale for using 45 years of age as a cutoff was based on analysis of the study cohort and the age of onset in inherited PHPT. The mean age of PHPT presentation ranges from 25 to 45 years in MEN1 patients [7], is 38 years in MEN2A patients [9], and 32 years in HPT-JT patients [8]. In addition, the incidence of PHPT increases with age, especially in women at the time of menopause (approximately age 45) and when postmenopausal [15]. Moreover, several studies have used 45 years as a cutoff to distinguish between young and old PHPT patients [10, 14]. In the ROC curve analysis of our study cohort, the specificity of a cutoff of 45 years old was 88% and the sensitivity was 55% for testing positive for a germline mutation. The low sensitivity could be due to patients diagnosed as having FIHP but who have a chance occurrence of PHPT in two first-degree relatives. Indeed, the average age of the FIHP group without positive germline mutations was 50 years old as compared to 38.7 years old for the other familial group with positive genetic testing ($n = 124$, $P < 0.001$). Additionally, the number of patients ≥ 45 years old in the sporadic group was only 12%.

Our data are consistent with the literature when reporting a higher female-to-male ratio in the sporadic group (3.8:1) and slightly higher in the familial group (1.6:1). In fact, one would expect an equal gender distribution in the familial group as MEN1, FIHP and JTS are inherited in an autosomal dominant pattern. However, the FIHP group might be contaminated with non-hereditary PHPT because of chance occurrence; indeed, in this subgroup (FIHP), the female to male ratio was 2.6:1 which may have skewed the gender ratio in the familial group.

The appropriate selection of patients for germline mutation testing is important and can have significant clinical ramifications for patients and their family members. The current study results estimate the probability, using readily available clinical information, that a patient may test positive. The percentage for testing positive for pathogenic variants in PHPT susceptibility genes for a patient with a family history of PHPT ranged from 6% to 92%. The percentage of having a positive germline mutation in a young patient (< 45 years old) with a positive family history ranged from 88% to 92% for patients with multigland disease and 34% to 45% for patients with single gland disease (Figure 1). Straker and colleagues

estimated the prevalence of germline mutations in disease-causing genes in young patients with PHPT (< 45 years old) with no known familial disease to be 9.3% [14]. Hence, the probability of genetic mutation increases significantly with a positive family history. On the other end of the spectrum, the probability of germline mutation was lowest for older males (10%) and females (6%) with family history and single gland disease.

There are no available data on the probability for testing positive for mutations PHPT susceptibility genes, unlike that which has been developed for breast cancer to justify genetic screening. According to the American Society of Clinical Oncology (ASCO) a probability > 10% of testing positive is an acceptable set point for use in selecting patients who may benefit from genetic testing for *BRCA1* and *BRCA2* mutations [16]. Thus, based on our model, and by analogy to the ASCO recommendations for patients with breast cancer, all patients with a family history of PHPT should be offered the opportunity for genetic counseling and testing.

The identification of germline mutation has significant implications, such as confirming the clinical diagnosis and, consequently, the implementation of the appropriate periodic clinical, biochemical, and radiological screening for associated tumors, as well as establishing the proper timing and extent of surgical intervention. For example, HPT-JT patients should undergo an immediate parathyroidectomy once the biochemical diagnosis of PHPT is established as they have a high risk of parathyroid carcinoma (37.5%) [8]. Most patients with MEN1 should have bilateral neck exploration and a subtotal parathyroidectomy instead of a focused parathyroidectomy, as they generally have multigland disease. Two-thirds of MEN1-related patient deaths are due to metastatic pancreatic neuroendocrine tumors and thymic carcinoid tumors, and the knowledge of patients having a *MEN1* germline mutation allows for screening and early treatment of these tumors when indicated [7].

The current study represents a comprehensive evaluation of germline mutation in known susceptibility genes for inherited PHPT as compared to sporadic PHPT. However, it has some limitations. For instance, patients with a family history of PHPT and negative genetic testing results may not have a familial disease but rather may have a chance occurrence of PHPT or a germline mutation in a yet-unidentified susceptibility gene. The age cutoff might result in missing older patients who have a genetic mutation. There were no patients with MEN2A in this study by design. PHPT is seldom the first and only manifestation in MEN2A, with most patients presenting with medullary thyroid carcinoma. Patients with no family history (the sporadic group) may have *de novo* cases of inherited PHPT, but the frequency of such cases would be low and unlikely to affect the predictive variable and probabilities of testing positive for a PHPT susceptibility gene mutation. Lastly, because we are a referral center for hereditary PHPT the clinical factors and probability of testing positive for a PHPT susceptibility gene may be different and need to be validated in different practice settings.

Conclusion

In summary, in addition to a family history of PHPT, male sex, age 45 years, and the presence of multigland disease should prompt physicians to offer the opportunity for genetic counseling and testing as it could influence the management and surveillance of PHPT.

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Figure 1.

Estimated percentage of testing positive for a germline mutation in a patients with a family history of primary hyperparathyroidism by age, sex, and presence of multigland disease. PHPT, primary hyperparathyroidism; MGD, multigland disease; SGD, single gland disease. Numbers above histogram represent percentage of patients with a germline mutation (*MEN1*, *CDC73*, or *GCM2*). Age cutoff or > 45 years.

Table 1

Clinical, Biochemical, and Parathyroid Disease Characteristics in Patients Undergoing Initial Parathyroidectomy for Primary Hyperparathyroidism

Variable	Family history of PHPT, n = 205	Sporadic PHPT, n = 452	p Value
Age, y, mean $\pm$ SD	42.8 $\pm$ 17.6	59.2 $\pm$ 13.5	< 0.001
Age $\geq$ 45 years, n (%)	113 (55.1)	55 (12.1)	< 0.001
Male sex, n (%)	80 (39)	95 (21)	< 0.001
Race/ethnicity, n (%)			
Caucasian	182 (88.8)	368 (81.4)	0.09
Asian	5 (2.4)	23 (5.1)	
African American	16 (7.8)	42 (9.3)	
Hispanic	1 (0.5)	15 (3.3)	
Multiracial	1 (0.5)	3 (0.6)	
Preoperative laboratory value, mean $\pm$ SD			
Total Ca, mmol/L	2.8 $\pm$ 0.26	2.77 $\pm$ 0.199	0.65
Intact PTH, pg/mL	145 $\pm$ 151	136 $\pm$ 113	0.96
No. of patients with >1 enlarged parathyroid gland, n (%)			
Multiple gland disease	144 (70.2)	65 (14.4)	< 0.001
Parathyroid carcinoma, n (%)	5 (2.4)	0 (0)	0.003
Follow-up, n (%) [*]			
Biochemical cure	183 (89.2)	435 (96.2)	0.005

Biochemical cure was defined as normal calcium and PTH during follow up

^{*} At 2 years follow-up

PHPT, primary hyperparathyroidism; PTH, parathyroid hormone.

Table 2

Results of Multivariable Logistic Regression Analysis for Germline Mutation Associated with Primary Hyperparathyroidism

Variable	Odds ratio	95% CI	p Value
Multigland disease *	14.1	8.9–22.5	<0.01
Age ≥ 45 years	7.74	4.7–12.8	<0.01
Male sex	1.68	1.02–2.77	0.041

* Defined as > 1 enlarged parathyroid gland.