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Hereditary Primary Hyperparathyroidism

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**Key Words:** genetic testing, primary hyperparathyroidism, familial hypocalciuric hypercalcemia (FHH), multiple endocrine neoplasia, familial isolated hyperparathyroidism (FIHP); MEN1, MEN2A, Hyperparathyroidism-Jaw Tumor syndrome (HPT-JT), CDC73

**Clinics Care Points**

- A thorough family history should be acquired in all patient presenting with PHPT, although the absence of any apparent relevant findings does not exclude the possibility of a hereditary disorder.

- Evaluating historic medical records to look for evidence of previously normal albumin corrected serum calcium levels may help differentiate between PHPT and FHH, with the latter associated with lifelong hypercalcemia.

- Close collaborative working between endocrinologists, clinical genetics and genetic laboratory scientists is required to facilitate the most appropriate genetic testing strategies and to facilitate accurate variant interpretation.

- Patients with hereditary PHPT syndromes including MEN1 and MEN2A should be managed by multidisciplinary teams with relevant expertise in the management of the respective disorders.
Key Points

- Hereditary forms of primary hyperparathyroidism (PHPT) account for up to 10% of cases of PHPT and include several multiple tumor syndromes or the occurrence of PHPT as an isolated endocrinopathy.

- Multiple tumor syndromes associated with hereditary PHPT include Multiple Endocrine Neoplasia Type 1 (MEN1), Type 2A (MEN2A), and Type 4 (MEN4) and the Hyperparathyroidism-Jaw-Tumor (HPT-JT) syndrome, each inherited in an autosomal dominant manner.

- Familial Isolated Hyperparathyroidism (FIHP) describes the familial occurrence of PHPT without the additional manifestations associated with hereditary PHPT syndromes. In the majority of FIHP kindreds the genetic basis of the disorder is undefined, although 15-20% of families harbor activating mutations in the GCM2 gene.

- Kindreds manifesting apparent FIHP, but harboring pathogenic variants in MEN1, CDC73 or CASR genes should be diagnosed and managed in accordance with the associated PHPT syndrome (i.e. MEN1, HPT-JT, Familial Hypocalciuric Hypercalcemia (FHH), respectively).

- It is important to distinguish PHPT from FHH, which has a similar biochemical phenotype to PHPT, but is characterized by hypocalciuria. Three forms of FHH are recognized, resulting from pathogenic variants in CASR (FHH1), GNA11 (FHH2) and AP2S1 (FHH3).

- Neonatal severe hyperparathyroidism (NSHPT) presents with severe hypercalcemia in early infancy, most commonly due to homozygous or compound heterozygous loss of function CASR variants.

- Genetic testing should be offered to patients in whom hereditary PHPT or FHH is suspected to improve clinical management and to identify other family members who may be at risk of disease.
Synopsis

Primary hyperparathyroidism (PHPT) is a commonly encountered clinical problem and occurs as part of an inherited disorder in ~10% of patients. Several features may alert the clinician to the possibility of a hereditary PHPT disorder (e.g. young age of disease onset) whilst establishing any relevant family history is essential to the clinical evaluation and will help inform the diagnosis. Genetic testing should be offered to patients at risk of a hereditary PHPT disorder, as this may improve management and allow the identification and investigation of other family members who may also be at risk of disease.
Introduction

Whilst the majority of patients presenting with primary hyperparathyroidism (PHPT) have a non-familial (i.e. sporadic) etiology, up to 10% of cases occur as part of a hereditary disorder, either as part of a multiple neoplasia syndrome or as an isolated endocrinopathy (Table 1).\textsuperscript{1-8}

Whilst identifying those with hereditary forms of PHPT may be challenging in the busy clinic, establishing a genetic diagnosis may have several benefits for the patient and wider family. These include ensuring the most appropriate management of the PHPT (e.g., determining the optimal surgical approach), facilitating the identification of any associated comorbidities, and allowing clinical evaluation and/or cascade testing of family members. Whilst several features in the clinical evaluation may indicate a higher likelihood of hereditary form of PHPT it is important to consider the possibility of such a diagnosis in all PHPT patients. Germline genetic testing forms an important part of the clinical assessment of those in whom a hereditary PHPT disorder is suspected, but requires judicious use to avoid potential diagnostic confusion and patient harms.\textsuperscript{9}

In this review, each of the major forms of hereditary PHPT is reviewed focusing on the parathyroid phenotype and briefly reviewing the molecular/genetic basis of the respective disorder. The hereditary forms of PHPT covered include: Multiple Endocrine Neoplasia Type 1 (MEN1);\textsuperscript{3,10,11} Multiple Endocrine Neoplasia Type 2A (MEN2A);\textsuperscript{8,12} Multiple Endocrine Neoplasia Type 4 (MEN4);\textsuperscript{13} Hyperparathyroidism-Jaw Tumor Syndromes (HPT-JT);\textsuperscript{2,14,15} and Familial Isolated Hyperparathyroidism (FIHP) (Table 1).\textsuperscript{5,16} A recently described disorder described as possible Multiple Endocrine Neoplasia Type 5, due to germline \textit{MAX} mutations, is not included as a clear association with PHPT has not been established.\textsuperscript{17} Given the overlapping clinical and biochemical features with PHPT (and the potential for diagnostic confusion) each of the Familial Hypocalciuric Hypercalcemic (FHH) disorders (i.e. FHH1-3) is reviewed,\textsuperscript{18,19} as is Neonatal Severe Hyperparathyroidism (NSHPT).\textsuperscript{1,19-22} Finally, a suggested clinical and genetic testing workflow is provided.
Clinical Evaluation: identification of patients with hereditary PHPT

History & Examination

The possibility of a hereditary disorder should be considered in all PHPT patients, and a failure to do so may lead to delays in establishing the correct diagnosis, suboptimal or inappropriate patient management, and a missed opportunity to identify other family members who may be at risk of disease. Although the clinical features in those with hereditary PHPT do not typically differ from those of sporadic PHPT, several features may alert the clinician to the possibility of a genetic disorder. For example, whilst sporadic PHPT most commonly affects those >50-years, with a 3:1 female predominance, hereditary forms of PHPT frequently have a younger age of onset with equal sex distribution (i.e., due to autosomal patterns of inheritance). Indeed, for children presenting with PHPT, the likelihood of a hereditary disorder approaches 50%, ~10% of adults with PHPT <40 years may have a hereditary cause.\(^1,2,23,24\)

During the initial evaluation of the patient, the presence (or history) of clinical manifestations associated with each of the respective hereditary syndromes should be established. Whilst PHPT is most frequently the presenting features of MEN1 and HPT-JT, in some instances it follows a preceding tumor (e.g., prolactinoma, insulinoma in MEN1). Likewise, in young patients presenting with PHPT, the clinical assessment should establish whether there are features of synchronous MEN-associated tumors. Similarly, a careful examination of the patient might provide diagnostic clues; for example, cutaneous manifestations such as facial angiofibroma or lipomas might indicate underlying MEN1;\(^3\) whilst more overt findings such as a concurrent neck mass or hypertension may indicate MEN2A (i.e. from medullary thyroid carcinoma or phaeochromocytoma, respectively).\(^8\) In addition, there should be a high clinical suspicion of a genetic diagnosis in those with recurrent or persistent biochemical features of PHPT following previous parathyroid surgery (i.e. raising the possibility of FHH or other hereditary PTH syndromes).
Elucidating the patient’s family history is paramount in identifying a potential hereditary disorder and should evaluate the presence of family members with a history of PHPT, or other MEN or HPT-JT-associated tumors. Indeed, a positive family history has been reported to be the strongest predictor of a genetic cause in those undergoing genetic testing for PHPT and/or FHH. However, several reasons may limit the availability of such information. For example, the patient may be unaware of their relatives’ medical history due to information not being shared between family members or geographical and/or social separation of the kindred. In addition, affected family members may be asymptomatic and remain undiagnosed, whilst certain disorders (e.g. HPT-JT due to CDC73 variants, FIHP due to GCM2 variants) are not fully penetrant such that some ‘affected’ family members (i.e., those harboring the disease-associated variant) may remain disease-free. Although the majority of familial PHPT disorders have an autosomal dominant mode of inheritance, in some instances neither parent is affected. For example, a proportion of MEN1, CDC73 and RET pathogenic variants occur de novo (i.e., appearing for the first time in the affected individual), whilst in rare instances there may be germline mosaicism, in which the disease-associated mutation occurs in a proportion of one parents’ gametes, such that apparently unaffected parents may give rise to multiple affected offspring. Recent studies report both germline and somatic mosaicism in kindreds with MEN1. In contrast a history of parental consanguinity may be evident in those with autosomal recessive disorders [e.g., NSPHT due to a homozygous calcium-sensing receptor (CASR) mutation].

Biochemical, Radiological and Pathological Evaluation

During the diagnostic work up, biochemical, and radiological features may help establish a potential genetic diagnosis. For example, the presence of longstanding mild hypercalcemia associated with inappropriately normal or elevated serum PTH and low 24-hour urinary calcium (e.g. <2.5mmol/l) and/or reduced calcium:creatinine clearance ratio (e.g. <0.01) may suggest FHH. Reviewing ‘historic’ medical records for evidence of a prior normal albumin-
corrected calcium may help clarify whether the hypercalcemia is acquired (i.e., in keeping in PHPT). The presence of multi-gland parathyroid involvement on pre-operative imaging or at the time of surgery (multi-gland hyperplasia or adenomas) should raise the possibility of a hereditary disorder, whilst the presence of parathyroid carcinoma or atypical parathyroid adenoma(s) may suggest HPT-JT.⁷
Hereditary Causes of Primary Hyperparathyroidism (PHPT)

Multiple Endocrine Neoplasia Type 1 (MEN1)

Definition/Epidemiology

MEN1 is an autosomal dominant disorder characterized by the presence of parathyroid, pituitary and pancreatic endocrine tumors, although additional tumors including thymic and bronchial carcinoids and adrenal cortical tumors are also observed.\textsuperscript{3,10,31} MEN1 has an estimated population prevalence of ~1 in 30,000 and results from heterozygous germline inactivating mutation of the \textit{MEN1} gene. A clinical diagnosis of MEN1 is made in a patient presenting with at least two of the three main clinical features, whilst a genetic diagnosis of MEN1 describes an individual harboring a known pathogenic \textit{MEN1} variant who may not yet manifest any clinical manifestations.\textsuperscript{3} Whilst PHPT in MEN1 is almost always benign, its early age of onset and almost universal occurrence may result in significant morbidity.\textsuperscript{3}

\textit{PHPT in MEN1: Clinical Features & Management}

Primary hyperparathyroidism occurs with almost complete penetrance (>95\%) in MEN1 patients, with equal sex distribution (M=F) and is the first manifestation of disease in 75-90\% of patients.\textsuperscript{3} Typically, PHPT presents in early adulthood with a mean age of onset of ~20 years, whilst >90\% of patients manifest PHPT by age 50 years. PHPT in MEN1 is unusual before 10 years of age, although asymptomatic and symptomatic cases have been reported as early as 4- and 8-years of age, respectively.\textsuperscript{32} Often the biochemical features of PHPT are mild and patients are frequently asymptomatic, although clinical presentations with symptomatic hypercalcemia or end organ involvement are reported (e.g. nephrolithiasis). Synchronous or asynchronous involvement of all 4 parathyroid glands is typically observed, whilst the histological appearances most commonly reveal chief cell hyperplasia although there is marked variation.\textsuperscript{7} Severe hypercalcemia is rare in MEN1, whilst parathyroid carcinoma has only occasionally been reported.\textsuperscript{3}
The treatment of MEN1-associated PHPT is controversial.\textsuperscript{3} Surgery is recommended in patients with symptomatic disease, severe hypercalcemia (i.e. corrected serum calcium >3.0 mmol/L), and/or evidence of end-organ damage (e.g. renal stones, osteoporosis).\textsuperscript{3} However, the timing and extent of surgery is debated, balancing the risks of persistent/recurrent PHPT with those of permanent hypoparathyroidism.\textsuperscript{33} Most centers advocate subtotal parathyroidectomy of at least 3.5 glands, which is associated with a reduced risk of permanent hypoparathyroidism (compared with total parathyroidectomy with auto-transplantation) but lower persistence/recurrence rates than lesser surgical approaches.\textsuperscript{3,33,34} For example, the removal of <3 glands is reported to be associated with persistence/recurrence rates of 15%-70%.\textsuperscript{3,33,34} However, some studies have reported more favorable short- to medium-term outcomes for MEN1 patients treated with less extensive surgical approaches (i.e. unilateral parathyroidectomy or minimally invasive parathyroidectomy), such that some centers advocate this approach in young MEN1 patients, accepting the likely need for future surgery but avoiding the immediate risks of permanent hypoparathyroidism.\textsuperscript{35} In those undergoing subtotal parathyroidectomy, concurrent bilateral transcervical thymectomy is recommended to remove parathyroid tumors that may be embedded within the thymus. Calcimimetics (e.g., cinacalcet), have been used to reduce or normalize serum calcium in MEN1 patients with PHPT in whom surgery has either failed or is contraindicated. Screening for PHPT in MEN1 patients with annual albumin-corrected serum calcium and PTH is recommended from age 5 years.\textsuperscript{3}

\textit{Genetics and Molecular Basis of MEN1-associated PHPT}

MEN1 is an autosomal dominant disorder due to loss of function germline variants of the \textit{MEN1} gene.\textsuperscript{3,10,36} The \textit{MEN1} gene is located on chromosome 11q13 and encodes the multifunctional protein Menin, Menin is predominantly a nuclear protein that acts as a molecular scaffold, facilitating the formation of several larger regulatory protein complexes involved in transcriptional and epigenetic regulatory activities as well as modulating multiple
cellular signaling pathways (e.g. Wnt/β-catenin, Hedgehog) in a cell-type and context-specific manner.\textsuperscript{37} In endocrine tissues Menin acts as a tumor suppressor protein, with MEN1-associated tumors including parathyroid tumors, typically demonstrating biallelic \textit{MEN1} inactivation (i.e. germline mutation of one \textit{MEN1} allele and somatic inactivation of the second allele).\textsuperscript{3}

More than 1,200 germline \textit{MEN1} mutations have been reported to date (~600 different mutations) and occur throughout the \textit{MEN1} coding region.\textsuperscript{36,38,39} \textit{De novo} \textit{MEN1} mutations are reported in 10\% of cases. Disease-causing \textit{MEN1} mutations include frameshift, nonsense, splice site and missense mutations and no clear genotype–phenotype correlation has been established. Approximately 10\% of patients with MEN1 will not have a mutation within the coding region of the \textit{MEN1} gene, and some of these cases will have large scale \textit{MEN1} deletions, or genetic alterations involving non-coding regions, whilst a minority will have pathogenic \textit{CDKN1B} variants (see MEN4).\textsuperscript{13,40} Notably, ~35\% of sporadic parathyroid adenomas reveal bi-allelic somatic inactivation of the \textit{MEN1} gene, further supporting the central role of the \textit{MEN1} gene in parathyroid tumorigenesis.\textsuperscript{41}
Multiple Endocrine Neoplasia Type 2A (MEN2A)

Definition/Epidemiology

Multiple Endocrine Neoplasia type 2A (MEN2A), is an autosomal dominant disorder with a reported incidence of ~1/80,000-live births, characterized by the occurrence of medullary thyroid carcinoma (MTC) in association with pheochromocytoma and parathyroid tumors. In contrast to MEN1 a clear genotype-phenotype correlation is observed such that the timing of MTC onset and likelihood of other clinical manifestations is related to the specific RET mutation.

PHPT Clinical Features & Management

PHPT occurs in <30% of patients with MEN2A, and typically presents in the 3rd to 4th decade. However, the risk of PHPT in MEN2A is related to the RET mutation. For example, 10-30% of patients with codon Cys634 mutations develop PHPT by 35-40 years of age, with carriers of the Cys634Arg RET variant at the highest risk. The age of onset of PHPT is variable and has been reported as young as 2 years of age. MEN2 patients with PHPT are frequently asymptomatic with mild hypercalcemia. The extent of parathyroid involvement varies from single gland involvement to synchronous or asynchronous involvement of multiple glands, with histological appearances ranging from mild enlargement with hyperplasia, to more overt abnormalities indistinguishable from parathyroid adenomas. The treatment of PHPT in MEN2A typically involves the removal of the enlarged/diseased parathyroid glands with the surgical approach adopted dependent on the timing of diagnosis relative to MTC.

Screening for PHPT in MEN2A is dependent on the specific RET mutation and recommended from age 11 years for those with the ATA ‘High’ risk (e.g. codon 634 variants), whilst it can be delayed until age 16 years for those with ‘moderate’ risk variants (e.g. codons 609-620).
Molecular genetics and RET Mutations

MEN2 results from germline mutation of the RET proto-oncogene, located at the pericentromeric region of chromosome 10q11.2, which encodes a single-pass transmembrane receptor tyrosine kinase (RTK) involved in neural crest and enteric nervous system development.\textsuperscript{48,49} RET signaling modulates the activity of multiple downstream signaling pathways (e.g. Ras/MAPK, PI3K/Akt, JNK, \(\beta\)-catenin/Wnt) that regulate diverse cellular processes including differentiation, proliferation, and cell migration.\textsuperscript{48,49}

More than 50 different germline RET mutations have been reported in association with MEN2A.\textsuperscript{8} The majority of MEN2A-associated RET mutations involve heterozygous non-synonymous amino acid substitutions of cysteine residues within the cysteine-rich extracellular domain, most frequently affecting codon Cys634, and result in ligand-independent dimerization and receptor activation, whereas MEN2A mutations affecting the intracellular domain (e.g. Tyr791, Val804, Ser891) result in activated monomers with autonomous tyrosine kinase activity.\textsuperscript{49}
Multiple Endocrine Neoplasia Type 4 (MEN4)

**Definition, Clinical Features and Management**

MEN4 is a rare autosomal dominant disorder with a similar clinical phenotype to MEN1, resulting from germline inactivating mutations of the *CDKN1B* gene.\(^{13}\) MEN4 should be considered in patients with a MEN1-like clinical phenotype in whom no *MEN1* mutation is identified,\(^{9}\) although to date, <40 MEN4 patients from <20 kindreds have been reported.\(^{13}\) PHPT together with functioning or non-functioning pituitary adenomas occur in 30-40% of patients, pancreatic and gastrointestinal NETS in 5-30%, whilst bronchial and cervical NETs, non-functional adrenal tumors, papillary thyroid cancer, lipomas, and breast cancer occur less frequently.\(^{13}\)

**PHPT Clinical Features & Management**

PHPT is reported in the majority (>90%), if not all, individuals identified with MEN4. The PHPT in MEN4 occurs at a relatively young age but later age than in MEN1 (typically ≥40 years), although has been reported as young as 15-years of age.\(^{13}\) Recurrent/persistent PHPT has been observed including in patients treated with subtotal parathyroidectomy indicating the involvement of multiple glands, and as such an investigation and treatment approach similar to MEN1 is suggested.\(^{13}\)

**Molecular Genetics**

The *CDKN1B* gene, located at chromosome 12p13, encodes the cyclin-dependent kinase inhibitor (CDKI) protein p27kip, which regulates the G1/S-phase checkpoint by binding to cyclin E/cdk2 to inhibit cell cycle progression. The observed germline *CDKN1B* mutations include protein-truncating (i.e. nonsense, frameshift, splice site) and missense variants, the majority resulting in reduced levels of p27\(^{kip}\) protein, or altered protein function. Loss of heterozygosity (LOH) is reported in MEN4-associated tumors including parathyroid adenomas
further supporting a tumor suppressor function. Germline \textit{CDKN1B} variants have been reported in patients with apparently sporadic parathyroid adenomas, although the relative high background frequency of rare non-synonymous variants in CDKI genes, indicates that some of these variants may represent benign alleles.\textsuperscript{50}
Hyperparathyroidism-Jaw Tumor Syndrome (HPT-JT)

Definition, Clinical Features and Management

The Hyperparathyroidism-jaw tumor (HPT-JT) syndrome is an autosomal dominant disorder characterized by the development of parathyroid tumors in association with ossifying fibromas of the maxilla and/or mandible, due to mutations of the cell cycle division 73 (CDC73) gene. In addition, some patients may develop uterine and renal tumors (e.g. Wilms’ tumors, papillary renal cell carcinomas), whilst rare reported manifestations include pancreatic adenocarcinomas, testicular mixed germ cell tumors, Hurthle cell thyroid adenomas and pituitary lactotroph adenomas. Notably, HPT-JT is associated with a high incidence of parathyroid carcinoma (PC). The ossifying fibromas in HPT-JT, which are reported in ~10-30% of patients, may be single or multiple, unilateral or bilateral and typically present in adulthood with treatment typically involving surgical removal. Notably, HPT-JT is associated with reduced disease penetrance such that ~10-35% of germline CDC73 mutation carriers do not manifest overt clinical features.

PHPT in HPT-JT; Clinical Features & Management.

PHPT in HPT-JT is reported to occur in 65-90% of patients and most frequently arises in early adulthood (median age of diagnosis 30-35 years), although is reported in children <10 years of age. The large majority (70-90%) of patients manifest a single parathyroid tumor, with only a minority (10-30%) having multi-gland involvement, although long term follow up studies indicate that asynchronous multi-gland involvement is more common. PC is reported in 15-20% of cases and has been reported as early as 8-years of age. Although HPT-JT associated parathyroid adenomas may be indistinguishable from sporadic parathyroid adenomas a higher frequency of cystic change and other distinct morphological are reported.

The investigation and treatment for the HPT-JT associated PHPT is similar to that in sporadic PHPT although early parathyroidectomy is advisable because of the increased occurrence of
PC. The possibility of HPT-JT and genetic testing for the presence of \textit{CDC73} mutations should be considered in all patients presenting with PC and atypical parathyroid adenomas.\textsuperscript{56}

\textit{Molecular Genetics and Genetic testing}

The \textit{CDC73} gene, located on chromosome 1q31.2, encodes the tumor suppressor protein parafibromin.\textsuperscript{14,15,57} More than 50 different germline heterozygous \textit{CDC73} mutations have been reported.\textsuperscript{2,14,15} Notably, somatic \textit{CDC73} mutations have also been reported in sporadic PC, parathyroid adenomas and ossifying fibromas. The majority of HPT-JT associated germline \textit{CDC73} mutations result in a functional loss of the parafibromin protein. Examination of parathyroid tumor tissue from HPT-JT patients frequently reveals loss of heterozygosity at the \textit{CDC73} locus (and loss of parafibromin expression), indicating a tumor suppressor function.\textsuperscript{15} Previous studies have demonstrated an over-representation of \textit{CDC73} mutations affecting exons 1, 2 and 7.\textsuperscript{2,15}

Parafibromin is an evolutionary conserved, predominantly nuclear protein, which forms a component of the ubiquitously expressed human RNA polymerase II-associated factor (PAF) complex, involved in transcription regulation. Parafibromin is an important regulator of several cell signaling pathways including Wnt, Notch and Hedgehog pathways.\textsuperscript{58} Furthermore, parafibromin, located within the cytoplasm, has been reported to control the stability of p53 mRNA thereby regulating apoptosis and is also a target of SUMOylation.\textsuperscript{59,60}
Non-Syndromic Familial PHPT

Familial Isolated Hyperparathyroidism (FIHP)

Definition/Epidemiology
Familial Isolated hyperparathyroidism (FIHP) refers to autosomal dominant familial hyperparathyroidism occurring as an isolated endocrinopathy, in the absence of clinical manifestations associated with other hereditary PHPT syndromes (e.g. MEN1, HPT-JT). Indeed, the diagnosis of FIHP should only be made after excluding the presence of mutations associated with other hereditary PHPT tumor syndromes (i.e. MEN1/MEN4, MEN2A, and HPT-JT) as well as FHH, and in this context large FIHP kindreds manifesting exclusive PHPT are rare. However, recent studies indicate that up to ~15-20% of FIHP kindreds harbor activating mutations in the GCM2 gene, located on chromosome 6, which encodes the chorion-specific transcription factor GCMB, involved in parathyroid gland development. The majority of FIHP-associated GCM2 variants reported to date occur within a 'C-terminal conserved inhibitory domain' and are associated with enhanced transcriptional activity in vitro. Furthermore, an increased prevalence of rare germline missense GCM2 variants has been reported in cohorts of patients with sporadic PHPT, although are associated with a low disease penetrance. Recent clinical studies report an increased prevalence of multi-gland parathyroid disease, lesser rates of surgical cure, and increased risk of parathyroid carcinoma in FIHP kindreds harboring GCM2 variants. The genetic basis of the remaining 80% of FIHP kindreds remains unexplained, although the majority of such kindreds have low numbers of affected individuals, and in some instances may represent the chance occurrence of sporadic disease in multiple family members. Indeed, to date, genetic investigation of such kindreds has not identified other recurrently mutated genes.
Familial Hypocalciuric Hypercalcemia (FHH)

Definition/Epidemiology

Familial Hypocalciuric Hypercalcemia (FHH) is a genetically heterogeneous disorder, typically characterized by lifelong mild-to-moderate hypercalcemia associated with inappropriately normal or elevated PTH, thereby mimicking the phenotype of PHPT. Clinically it is differentiated from PHPT by the finding of a low urinary calcium excretion (e.g. calcium-creatinine clearance ratio (CCCR) of <0.01; or 24 hour urinary calcium <2.5mmol) as ~80% of FHH patients are hypocalciuric. However, there may be considerable biochemical overlap with PHPT, as ~20% of FHH patients have a CCCR above this threshold, whilst 10-20% of PHPT patients have a CCCR below this cut-off. Thus, genetic testing is increasingly used in the diagnostic evaluation. To date, three variants of FHH are identified each inherited in an autosomal dominant manner; FHH1 represents ~65% of cases and is due to loss of function mutations of the CASR; FHH2 accounts for <5% of FHH cases and is due to loss of function GNA11 mutations; and FHH3, which accounts for ~20% of FHH cases without a CASR mutations, is due to loss of function mutations in the AP2S1 gene. FHH was previously considered a rare disease, although recent population genetic studies indicate a prevalence of 1/1000-1/5000. The importance of recognizing FHH from PHPT (and other causes of hereditary PHPT) is in the avoidance of unnecessary investigation and/or treatment. Indeed, ~10-25% of patients who have undergone failed neck exploration for apparent PHPT, have been reported to have FHH.

Clinical features & Management

Although there may be subtle difference in phenotype, each of the recognized forms of FHH are generally indistinguishable. The majority of patients are asymptomatic although a minority report symptomatic hypercalcemia (e.g. fatigue, muscle cramps, constipation), whilst end-organ manifestations are observed in a small percentage of patients (e.g. nephrocalcinosis,
osteoporosis and/or fractures, chondrocalcinosis, pancreatitis), although it is unclear if such associations are causative.\textsuperscript{18} Whilst most individuals with FHH1 and FHH2 have a mild phenotype, individuals with FHH3 typically manifest a higher serum calcium, and are reported to have an increased likelihood of reduced bone mineral density, osteomalacia, recurrent pancreatitis and cognitive dysfunction.\textsuperscript{1,7,19}

Asymptomatic individuals with FHH do not typically require treatment. For those with symptomatic hypercalcemia or potential complications related to the condition (e.g. recurrent pancreatitis), treatment with cinacalcet has been associated with improvement in biochemical parameters (e.g. normalization of serum calcium) in each of FHH1-3.\textsuperscript{18,19,67} Although the parathyroid glands of patients with FHH may be mildly enlarged with or without hyperplasia, surgery including subtotal parathyroidectomy is generally ineffective and should be avoided.\textsuperscript{7,18} Parathyroid adenomas have been reported in occasional patients with apparent inactivating \textit{CASR} mutations, where surgery has improved hypercalcemia, although it is unclear whether these associations are causal.\textsuperscript{68}

A history of parental FHH may be important for infants in the neonatal period as they may be at risk of calcium-related phenotypes. For example, infants who inherit a paternal FHH mutation and are exposed to normal maternal calcium levels \textit{in utero}, may manifest marked hypercalcemia (e.g. manifesting as neonatal hyperparathyroidism (see below)), whereas the offspring of affected mothers who do not inherit the FHH mutation may manifest transient neonatal hypoparathyroidism following exposure to relatively high maternal calcium levels during pregnancy.\textsuperscript{1,69}

\textit{FHH1-3: Molecular Genetics}

\textit{FHH1}: FHH1 is an autosomal dominant disorder due to germline heterozygous mutations in \textit{CASR} gene (a small minority result from homozygous \textit{CASR} mutations), located on chromosome 3q21, that encodes the calcium sensing receptor (CaSR).\textsuperscript{19,22,65} The CaSR is a
class C G-protein coupled receptor (GPCR), localized to the cell membrane within calcitropic tissues (e.g. parathyroid glands, kidney, bone) and is primarily responsible for regulation of PTH secretion and urinary calcium excretion. Around 300 different CASR mutations have been reported in FHH1 patients, the majority missense variants (>80%), with the remainder loss of function variants. The majority of missense variants are located within the extracellular domain, impacting key functional domains) with the remainder located within the transmembrane domain. Recent population-based genetic studies suggest FHH1 occurs with a prevalence of ~75/100,000 individuals.

**FHH2:** FHH2 results from germline heterozygous loss-of-function variants in the GNA11 gene, located on chromosome 19p13.3. GNA11 encodes the Gα11 protein, which forms part of the heterotrimeric G-protein complex associated with CaSR signaling. To date, only a small number of FHH2-associated GNA11 mutations have been reported, including several missense variants and one in-frame deletion, which impair CaSR signaling by disrupting key functional domains involved in coupling with the CaSR or interacting with downstream effector proteins.

**FHH3:** FHH3 results from germline loss of function mutations of the AP2S1 gene located on chromosome 19q13.3, which encodes the adaptor protein 2 sigma (AP2σ) subunit that forms part of a larger heterotetrameric complex involved in clathrin-mediated endocytosis. In contrast to FHH1 and FHH2, the overwhelming majority of FHH3 associated mutations affect a single amino acid residue, Arg15, although several different amino acid substitutions have been observed (e.g. Arg15Cys, Arg15His, Arg15Leu). These different Arg15 variants are predicted to disrupt the interaction between the AP2 complex and the CaSR intracellular domain, thereby reducing endocytosis of the receptor, and altering cell surface expression and signal transduction.
Neonatal Severe Hyperparathyroidism (NSHPT)

Clinical Features and Management

NSHPT is a rare disorder that typically presents in the post-partum period (median age of diagnosis 14 days) with severe hypercalcemia (serum calcium typically 3.0-6.0mmol/L), elevated parathyroid hormone, skeletal demineralization leading to fracture, respiratory distress, failure to thrive and if left untreated is potentially fatal.\(^1\,^{20}\,^{22}\,^{74}\) Infants with NSHPT typically manifest markedly enlarged parathyroid glands with evidence of diffuse chief cell hyperplasia.\(^7\) Most commonly, NSPHT results from homozygous or compound heterozygous loss of function CASR variants, although may also occur in the setting of heterozygous CASR mutations.\(^20\,^{74}\) Indeed some centers differentiate between NSPHT and neonatal hyperparathyroidism (NHPT) on the presence of homozygous/bi-allelic or heterozygous CASR mutations, respectively, and that in the latter group, the severity of hypercalcemia is typically less pronounced than in NSHPT, and may improve over time to a phenotype consistent with FHH1, without the need for parathyroid surgery.\(^1\,^{20}\,^{74}\) The treatment of NSHPT is usually with urgent parathyroidectomy, with bisphosphonates employed pre-operatively to control the hypercalcemia. Cinacalcet, has also been used successfully to lower calcium and PTH levels in some NSHPT patients including those with heterozygous (e.g. Arg185Gln) and homozygous (e.g. Arg69His) CASR mutations, although it is important to note that some mutations are unresponsive to such therapy.\(^74\)
Genetic testing workflow

The decision to undertake genetic testing should be determined by the potential to improve health outcomes in the patient and/or wider family (Figure 1). For example, establishing a genetic diagnosis of a multiple tumor syndrome such as MEN1, MEN2A or HPT-JT not only facilitates the appropriate investigation and management of the associated clinical features in a patient but also enables predictive testing in family members. Genetic testing may also resolve diagnostic confusion arising from phenocopies (i.e. patients manifesting the phenotypic characteristics of a particular genetic disorder, but without the relevant gene mutation) which are reported in clinical presentations of MEN1, MEN2A, HPT-JT and FHH. However, genetic testing, it is not without potential harms. For example, uncertain test results or variant misclassification (e.g. benign variants reported as pathogenic) may lead to diagnostic confusion and/or inappropriate patient management.

Although a number of criteria for genetic testing in patients with PHPT have been suggested (Table 2), these have not been evaluated systematically. Likewise, the genetic testing strategy employed will depend on several factors, although the majority of centers now adopt disease-targeted gene panels to facilitate the simultaneous evaluation of multiple PHPT and/or FHH genes (Table 2, Figure 1). The evaluation of variants identified during genetic testing should be undertaken using standardized methods (e.g. American College of Medical Genetics and Genomics (ACMG) Guidelines) (Figure 1), which combine a number of variant and gene-specific factors to categorise variants into one of five categories: benign, likely benign, variant of uncertain significance (VUS), likely pathogenic, or pathogenic. However, this classification system is not absolute, and is dependent on the accuracy of available evidence at the time of assessment (Figure 1). Furthermore, a high cumulative frequency of rare coding region variation in the background population may hamper variant interpretation and increases the potential to identify VUS variants during gene panel testing.
Following genetic testing it is important to consider the result in the clinical context of the patient (i.e. to establish a 'clinical-genetic' diagnosis). Where a genetic diagnosis is suspected but initial genetic testing is negative it is important to liaise with the local genetics team to determine whether alternate testing strategies may be helpful. For example, next-generation sequencing methods with increased depth of sequencing, have identified \textit{MEN1} mutations in patients in whom prior Sanger genetic testing was negative.\textsuperscript{30,76} Finally, following the identification of a positive test result in a patient (i.e. pathogenic/likely pathogenic variant), clinical evaluation and predictive testing of family members should be undertaken through the clinical genetics team (Figure 1).
Summary

Up to 10% of PHPT cases occur in a hereditary context either as part of one of several syndromic disorders or as an apparently isolated feature. Differentiating these hereditary causes is important not only to ensure the appropriate management of PHPT, but to identify any co-existent clinical features in patients with hereditary PHPT syndromes as well as allowing the identification of other affected family members. Germline genetic testing forms a key component of the evaluation of patients with possible hereditary PHPT but should only be undertaken after thorough clinical evaluation.
References


42. Machens A, Dralle H. Therapeutic Effectiveness of Screening for Multiple Endocrine Neoplasia Type 2A. *J Clin Endocrinol Metab.* 2015;100:2539-2545.


Figure 1. Illustrative workflow for genetic testing in patients presenting with PHPT/FHH phenotypes

The strategy for genetic testing (i.e. single gene testing vs disease-targeted gene panel) will be determined by the clinical presentation and local policies. Variants identified through genetic testing should be classified according to the American College of Medical Genetics and Genomics (ACMG) guidelines. Where variants of uncertain significance (VUS) are identified, it is important to establish whether additional information may be acquired that will facilitate a more categorical classification (e.g. genetic testing of further affected family members to evaluate for co-segregation of the variant with disease phenotype, additional *in vitro*/*in silico* functional analysis). Whether patients harboring VUS variants require clinical follow up may depend on the further risk stratification of the variant (e.g. ‘hot’ vs ‘cold’ VUS classification). Furthermore, it should be noted that variant classification may change over time such that periodic re-evaluation of variants is recommended.

Abbreviations: FDRs, first-degree relatives; MLPA, multiplex ligation-dependent probe amplification.
### Table 1. Syndromic and non-syndromic conditions associated with PHPT

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chrom. Pos</th>
<th>Inherit.</th>
<th>Variant type</th>
<th>Notes on disease-associated variants/PHPT phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Syndromic forms of Hereditary PHPT</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Multiple Endocrine Neoplasia Type 1 (MEN1)</td>
<td>MEN1</td>
<td>11q13.1</td>
<td>AD</td>
<td>ms, LOF, del&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Multiple Endocrine Neoplasia Type 2A (MEN2A)</td>
<td>RET</td>
<td>10q11.21</td>
<td>AD</td>
<td>ms</td>
</tr>
<tr>
<td>Multiple Endocrine Neoplasia Type 4 (MEN4)</td>
<td>CDKN1B</td>
<td>12p13.1</td>
<td>AD</td>
<td>ms, LOF, del&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hyperparathyroidism Jaw Tumor (HPT-JT) Syndrome</td>
<td>CDC73</td>
<td>1q31.2</td>
<td>AD</td>
<td>ms, LOF, del&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Non-Syndromic Forms of Hereditary PHPT including FHH</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Familial Isolated Hyperparathyroidism (FIHP)</td>
<td>GCM2</td>
<td>6p24.2</td>
<td>AD</td>
<td>ms,</td>
</tr>
<tr>
<td>(MEN1&lt;sup&gt;*&lt;/sup&gt;)</td>
<td>11q13.1</td>
<td>AD</td>
<td>ms, LOF</td>
<td>FIHP kindreds with pathogenic MEN1 variant should be classified as MEN1</td>
</tr>
<tr>
<td>(CDC73&lt;sup&gt;*&lt;/sup&gt;)</td>
<td>1q31.2</td>
<td>AD</td>
<td>ms, LOF</td>
<td>FIHP kindreds with pathogenic CDC73 variant should be classified as HPT-JT.</td>
</tr>
<tr>
<td>(CASR&lt;sup&gt;*&lt;/sup&gt;)</td>
<td>3q13.33-q21.1</td>
<td>AD</td>
<td>ms, LOF</td>
<td>It is unclear whether kindreds reported to have FIHP due to CASR mutations represent a distinct entity from FHH1</td>
</tr>
<tr>
<td>Familial Hypocalciuric Hypocalcemia Type 1 (FHH1)</td>
<td>CASR</td>
<td>3q13.33-q21.1</td>
<td>AD</td>
<td>ms, LOF</td>
</tr>
<tr>
<td>Familial Hypocalciuric Hypocalcemia Type 2 (FHH2)</td>
<td>GNA11</td>
<td>19p13.3</td>
<td>AD</td>
<td>ms, (if-del)</td>
</tr>
<tr>
<td>Familial Hypocalciuric Hypocalcemia Type 3 (FHH3)</td>
<td>AP2S1</td>
<td>19q13.32</td>
<td>AD</td>
<td>ms (Arg15)</td>
</tr>
<tr>
<td>Neonatal Hyperparathyroidism (NHPT)</td>
<td>CASR</td>
<td>3q13.33-q21.1</td>
<td>AD</td>
<td>ms, LOF</td>
</tr>
</tbody>
</table>

<sup>a</sup>Although kindreds with FHH have been identified to harbor MEN1, CDC73 and CASR mutations, these likely represent incomplete/atypical expression of the associated syndromic PHPT/FHH disorder. Typically, those harboring pathogenic/likely pathogenic variants in MEN1 and CDC73 genes should be classified as having MEN1, and HPT-JT, respectively. Although a small number of individuals/kindreds with inactivating CASR mutations have been reported to have features more typical of PHPT (i.e. hypercalciemia, hypercalciuria and parathyroid adenoma/hyperplasia), most have a biochemical phenotype more typical of FHH, which is not improved with surgery.

<sup>*Several monogenic disorders may result from partial or whole gene deletions, which may not be detected by single-gene or gene-panel testing. In this setting, alternate methods may be required including MLPA, aCGH or FISH. Some NGS sequencing platforms including those used in gene-panel testing may also detect these large-scale deletions. Loss of function (LOF) variants include nonsense mutations (i.e. resulting from a SNV introducing a premature stop codon), small insertions or deletions (indels) most frequently resulting in a frameshift in the coding sequence and early introduction of a stop codon, and those affected canonical splice sites resulting in aberrant transcript processing. Abbreviations: ECD, extracellular domain; TM, transmembrane domain; ICD, intracellular domain; ms, missense; LOF, loss of function; hom, homozygous; comp het, compound heterozygous; AD, autosomal dominant; AR, autosomal recessive; del, deletion (indicating large scale whole or partial gene deletion); if-del, in-frame deletion; Chrom Pos, chromosomal position; Inherit, inheritance.</sup>
Table 2 Potential indications for genetic testing in patients with PHPT/FHH

<table>
<thead>
<tr>
<th>Indication for Genetic testing</th>
<th>Genetic Testing strategy</th>
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<tbody>
<tr>
<td>All children/young persons with PHPT phenotype (e.g. &lt;21-years of age)</td>
<td>PHPT/FHH panel(^a) (including (\text{MEN1/CDC73/CASR}))</td>
</tr>
<tr>
<td>Adults with PHPT &lt;40 years(^b) of age</td>
<td>PHPT panel (FHH panel(^b))</td>
</tr>
<tr>
<td>Multi-gland PHPT (adenoma or hyperplasia)</td>
<td>PHPT panel (FHH panel(^b))</td>
</tr>
<tr>
<td>Parathyroid Carcinoma</td>
<td>(\text{CDC73 (GCM2(^*))})</td>
</tr>
<tr>
<td>Atypical parathyroid adenoma(s)</td>
<td>(\text{CDC73 (GCM2(^*))})</td>
</tr>
<tr>
<td>Clinical diagnosis and/or features consistent with hereditary PHPT syndrome (current or past history of relevant tumors)</td>
<td>Relevant gene(s) or PHPT gene panel</td>
</tr>
<tr>
<td>- MEN1/4 (e.g. PHPT + other MEN1 associated tumor(s))</td>
<td>(\text{MEN1/CDKN1B})</td>
</tr>
<tr>
<td>- MEN2A (e.g. PHPT + MTC and/or pheochromocytoma)</td>
<td>(\text{RET})</td>
</tr>
<tr>
<td>- HPT-JT (e.g. PHPT + ossifying fibroma of mandible/maxilla)</td>
<td>(\text{CDC73})</td>
</tr>
<tr>
<td>Family history suggestive of hereditary PHPT syndrome (i.e. manifestations consistent with MEN1/4, MEN2A or HPT-JT)</td>
<td>Relevant gene (PHPT panel)</td>
</tr>
<tr>
<td>Family history of PHPT/FHH in FDRs</td>
<td>PHPT/FHH panels</td>
</tr>
<tr>
<td>History and biochemistry consistent with FHH (e.g. CCCR&lt;0.01)</td>
<td>FHH panel</td>
</tr>
<tr>
<td>Previous negative neck exploration/non-curative surgery</td>
<td>FHH panel (PHPT panel)</td>
</tr>
</tbody>
</table>

This list of indications above is not evidence-based but rather provided to help highlight patients with a potentially increased risk of hereditary disease. The criteria for referral in any given center will depend on local policies and resources.

\(^a\)To date no clear ‘age-alone’ cut-off has been established for genetic testing. In some centers the age cut-off is dependent on the presence of additional risk factors, e.g. presence of multi-gland involvement, or positive family history.

\(^b\)Although genetic testing of \(\text{CDC73}\) is recommended in those with parathyroid carcinoma/atypical parathyroid adenomas, a recent study reports an increased prevalence of parathyroid carcinoma in those with \(\text{GCM2}\) mutation.

\(^c\)Most centers now adopt the use of disease targeted gene panels for investigation of hereditary PHPT and FHH syndromes. The content of the panels will vary by center but typically includes: PHPT panel; \(\text{CDC73, CDKN1B, MEN1, RET, CASR, GCM2}\); FHH panel; \(\text{AP2S1, CASR, GNA11}\). In some centers these are combined into a single PHPT/FHH panel. The value of genetic testing for pathogenic variants in \(\text{GCM2}\) has not yet been fully evaluated as variants are likely associated with low disease penetrance. Abbreviations: FDR, first-degree relative; FHH, familial hypocalciuric hypercalcemia; PHPT, primary hyperparathyroidism; HPT-JT, Hyperparathyroidism-Jaw tumor syndrome; MEN1/4, Multiple Endocrine Neoplasia Type 1/Type 4; MEN2A, Multiple Endocrine Neoplasia Type 2A; CCCR, calcium creatinine clearance ratio.