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Familial Syndromes of Primary Hyperparathyroidism

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Abstract

Regulation of serum calcium in vertebrates is maintained by the actions of the parathyroid glands working in concert with vitamin D and critical target tissues that include the renal tubules, the small intestine, and bone cells. The parathyroid glands release parathyroid hormone (PTH) into the systemic circulation as is required in order to maintain the serum calcium concentration within a narrow physiologic range. Excessive secretion of PTH from one or more abnormal parathyroid glands however results in primary hyperparathyroidism (HPT), a metabolic disease typically associated with abnormally elevated serum calcium. Although HPT is typically a sporadic disease, it can represent a manifestation of an inherited syndrome. Many sporadic parathyroid tumors result from inactivating mutations in tumor suppressor genes that were first discovered by the analysis of genomic DNA from patients with HPT as part of an inherited syndrome. Somatic and inherited alterations in DNA encoding proto-oncogenes can also cause parathyroid neoplasia. Two promising future approaches for the discovery of novel genes pertinent to parathyroid tumor development are the analysis of acquired genetic alterations in DNA isolated from parathyroid tumors and the investigation of familial HPT in kindreds lacking germline mutation in the known genes predisposing to HPT.

Keywords: multiple endocrine neoplasia, MEN1, MEN2A, jaw tumor syndrome, CDC73, HRPT2, GCM2, CCND1, RET, CASR, CDKN1B, tumor suppressor, oncogene

1. Introduction

The inappropriate or excessive secretion of parathyroid hormone (PTH) from one or multiple abnormal parathyroid glands typically results in hypercalcemia and the disorder of mineral metabolism called primary hyperparathyroidism (HPT) [1]. Most cases of HPT are sporadic (~95%). Among the small remaining fraction of patients with an inherited basis for HPT, most harbor germline mutation of a known parathyroid tumor susceptibility gene (listed in **Table 1**). In spite of their infrequency, study of the genetics of these uncommon inherited syndromes has yielded substantial insight into the etiology of both sporadic and familial parathyroid tumor development. Since the release of PTH from parathyroid cells involves close regulation by the calcium-sensing receptor (CASR), a cell surface transmembrane receptor of the G protein-coupled receptor family C [2], the germline mutation of the CASR and other genes mediating its signaling can also result in inherited syndromes characterized by hypercalcemia and circulating levels of PTH that are elevated or inappropriately normal. This chapter will summarize current knowledge

Gene	Corresponding protein	Chromosomal location	Associated hyperparathyroid syndrome: main syndromic manifestations	Features of syndromic parathyroid tumors
MEN1	Menin	11q13.1	Multiple endocrine neoplasia type 1 (MEN1): anterior pituitary, parathyroid, enteropancreatic, foregut carcinoid tumors	Multiple, asymmetric tumors typical (>99% benign)
CDC73/ HRPT2	Parafibromin	1q31.2	Hyperparathyroidism-jaw tumor syndrome: fibro-osseous jaw, parathyroid, uterine tumors; renal cysts	Single tumor common (~20% malignant)
CDKN1B	P27(Kip1)	12p13.1	Multiple endocrine neoplasia type 4 (MEN4): anterior pituitary, other involvement varies	Single to multiple glands (benign in reports to date); can be recurrent
GCM2	Glial cells missing transcription factor 2	6p24.2	Familial isolated primary hyperparathyroidism	Single to multiple glands
CASR	Calcium-sensing receptor	3q13.33-q21.1	Familial hypocalciuric hypercalcemia type 1 (FHH1) with heterozygous inactivation; neonatal severe hyperparathyroidism (NSHPT) with homozygous inactivation	FHH1: near-normal size and surgical pathology; altered serum calcium set-point for PTH release NSHPT: marked enlargement of multiple glands by polyclonal (non-neoplastic) mechanism
GNA11	G protein α11 subunit	19p13.3	Familial hypocalciuric hypercalcemia type 2 (FHH2)	ND
AP2S1	Adaptor protein-2 sigma subunit	19q13.32	Familial hypocalciuric hypercalcemia type 3 (FHH3): hypercalcemia more severe than in FHH1	ND
RET	c-Ret	10q11.21	Multiple endocrine neoplasia type 2A: medullary thyroid cancer, pheochromocytoma, parathyroid tumors	Single tumor common (>99% benign)
CCND1/ PRAD1	Cyclin D1	11q13.3	NA (to date, only implicated in sporadic parathyroid tumors)	NA (to date, only implicated in sporadic parathyroid tumors)

Table 1.
Genes implicated in syndromic parathyroid neoplasia and related hypercalcemic states.

of the clinical genetics and molecular pathophysiology of HPT that results from both benign and malignant parathyroid gland neoplasia.

2. The evolution of calcium regulation in vertebrates

In sea water the concentration of elemental calcium is approximately 10 mM. As a result, early eukaryotes living in a marine environment had easy access to calcium. Given this abundant supply of extracellular calcium, numerous intracellular processes evolved in simple eukaryotes that depended on this divalent cation. Such calcium-dependent processes were preserved in metazoans. Thus marine chordates and early vertebrate fish depended on calcium for cellular processes such as membrane permeability, neurotransmitter release, intracellular second messenger signaling, muscular contraction, neuromuscular excitability, and the actions of multiple calcium-dependent enzymes. Calcium's particular coordination chemistry facilitated many proteins' ability to reversibly bind divalent calcium ions, thus enabling signaling through such binding [3].

Calcium is much scarcer on land compared to the marine environment. As lobe-finned fish, marine vertebrates believed to be the ancestors of the early amphibians, began to explore the periphery of the terrestrial environment, evolutionary pressure to develop a system of internal calcium balance mounted. A system of internal calcium homeostasis at the organismal level would ensure the continued preservation and function of numerous cellular and tissue operations that vitally depended on calcium.

Metabolically-active trabecular or cancellous bone in lobe-finned fish and associated hematopoietic bone marrow likely co-evolved [4]. These developments probably both lightened overall skeletal mass and provided a reliable internal source of calcium as a basis for calcium homeostasis. The lightening of skeletal mass was critical since lobe-finned fish and early amphibians had to come to terms with full gravitational force in their terrestrial movements, no longer buoyed by surrounding seawater in accordance with Archimedes' principle [5]. The potential significance of the close physical apposition of hematopoietic bone marrow to spongiform bone, inferred from X-ray synchrotron microtomography of fossilized lobe-finned fish humerus [4], is suggested by the realization that osteoclasts, cells uniquely specialized to mobilize ionized calcium via resorption of bone, develop from hematopoietic stem cell precursors [6]. In contrast, osteoblasts, which lay down osteoid and mineralize bone, derive from mesenchymal stem cells which are abundant in non-hematopoietic bone marrow.

Although analogs of Gcm2, Gata3, CaSR, PTH, and other genes associated with the development and function of human parathyroid glands are expressed in the fish gills, actual parathyroid glands are first seen in amphibians [7–9]. Complete surgical excision of parathyroid gland tissue in amphibians, reptiles, birds, and mammals results in tetany and death.

3. The pathophysiology of primary hyperparathyroidism

PTH secretion from cells of the parathyroid glands is finely regulated in response to changes in the ambient ionized calcium level in order to maintain the circulating calcium concentration within a defined physiologic range. The G protein-coupled CASR is a critical regulator of PTH secretion and is located on the plasma membrane of chief cells in the parathyroid glands [10, 11]. In a classic endocrine negative feedback loop, the active form of cholecalciferol,

1,25-dihydroxyvitamin D, whose synthesis is stimulated by PTH acting on proximal renal tubular cells, inhibits PTH biosynthesis and release from parathyroid cells [12–15]. The simultaneous demonstration of elevated serum calcium with an inappropriately normal or elevated PTH is a typical clinical definition of HPT [16]. The vast majority of parathyroid tumors are adenomas (i.e. benign tumors), with parathyroid cancer accounting for less than 1% of HPT in most series.

Most cases of HPT are sporadic with inherited forms of HPT representing only 2–5% of cases. As illustrated in **Table 1**, research into the molecular pathophysiology of this small subcategory of cases has notwithstanding yielded important understanding with respect to the genes and pathways that promote parathyroid tumorigenesis. Multiple endocrine neoplasia type 1 (MEN1), multiple endocrine neoplasia type 2A (MEN2A), the hyperparathyroidism-jaw tumor syndrome (HPT-JT), and familial isolated hyperparathyroidism (FIHP) are the most common inherited disorders associated with HPT [17–21]. Familial hypocalciuric hypercalcemia (FHH) is a related and largely benign autosomal dominant condition characterized by lifelong asymptomatic hypercalcemia. Often mis-diagnosed as HPT, in FHH the PTH-dependent hypercalcemia does not correct with partial or even subtotal parathyroidectomy [22]. The relevance of these inherited disorders to the underlying molecular pathogenetic alterations in parathyroid tumorigenesis will be discussed in more detail below.

4. Oncogenes and proto-oncogenes

Mutant genes that drive cell growth are called oncogenes and represent one potential molecular mechanism for tumor development. Oncogenes are mutationally activated versions of naturally occurring genes, called proto-oncogenes, which under normal conditions positively regulate cell division and/or cell growth [23]. Oncogenes represent gain-of-function mutants or overexpressed forms of proto-oncogenes that can induce cell growth and cell division, often in a tissue-specific fashion, resulting in tumor formation. Proto-oncogenes often encode proteins that are involved in mitogenic signal transduction. In the context of currently recognized familial cancer syndromes, germline mutational activation of proto-oncogenes is rare as an etiology compared to the inactivation of tumor suppressor genes (see below). Constitutive proliferative signaling resulting from the germline activation of most proto-oncogenes would presumably be deleterious to embryonic and fetal development.

5. The role of tumor suppressor genes in tumor development

Alfred Knudson proposed another model for tumor development based on the study of retinoblastoma disease patterns nearly 50 years ago [24]. Sporadic retinoblastoma is usually monocular. Familial retinoblastoma, though rare compared to the sporadic form, is more frequently binocular and has a much earlier age of onset. The "two-hit" hypothesis of tumor development, as proposed by Knudson, hypothesizes that two events (or "hits") in a parental cell confer a selective growth advantage and result in that cell's clonal expansion [25].

Newer clinical and molecular genetic insight that has emerged since his original proposal allow us to update Knudson's concept. In many hereditary tumor syndromes, an inherited germline DNA mutation that affects one copy of a tumor suppressor gene represents the first "hit" or event and is present throughout all cells of the affected offspring. The greater likelihood of any particular cell acquiring a

"second hit", i.e. a somatic mutation in the second allele of the same tumor suppressor gene that was heretofore unaffected, accounts for the earlier age of onset and predisposition for bilateral and multifocal disease in hereditary tumor syndromes. This "second hit" in somatic DNA, that disables the remaining wild-type allele, typically results from a deletion that involves a portion or the entirety of a chromosome. In the familial tumor syndromes MEN1 and HPT-JT, inactivating mutation that involves both alleles of the *MEN1* and the *CDC73/HRPT2* tumor suppressor genes, respectively, can often be found in parathyroid tumor-derived DNA. In such patients, the first "hit", namely a loss-of-function mutation of the relevant tumor suppressor gene, can frequently be demonstrated in the germline DNA.

6. Multiple endocrine neoplasia type 1 (MEN1)

MEN1 is the most common hereditary cause of primary hyperparathyroidism [26]. The syndrome of MEN1 is characterized by the predisposition to develop tumors derived from cells in the anterior pituitary, parathyroid glands, and endocrine cells present in the gut and pancreatic islets (such as gastrinomas, and pancreatic neuroendocrine tumors such as insulinomas) [27]. Tumors in several other endocrine organs and non-endocrine tumors such as lipomas, angiofibromas, and leiomyomas affecting the esophagus, uterus, and/or ureters for example, can also be associated with the syndrome [27]. HPT is the most penetrant hormonal feature of MEN1.

Familial MEN1 is characterized by autosomal dominant transmission. The predisposition to tumor development in one of the tissues characteristically involved in the MEN1 syndrome is caused by germline inactivating mutation in one copy of the *MEN1* gene on chromosome 11q13 [28]. As of 2015, 576 unique germline mutations in *MEN1* were reported from patients and families with MEN1 [29]. The study of DNA derived from pituitary, parathyroid, and entero-pancreatic tumors from MEN1 patients has shown that most syndromic tumors possess an acquired deletion or other inactivating mutation of the second, wild-type *MEN1* allele [18, 30]. Approximately 10% of patients with MEN1 on a clinical basis are germline *MEN1* mutation-negative.

Conventional DNA sequencing of tumor DNA has identified somatic *MEN1* mutation in up to 35% of sporadic parathyroid adenomas [31–35]. In studies testing for loss-of-heterozygosity (LOH) in sporadic parathyroid adenomas, the frequency of LOH at the *MEN1* locus on chromosome 11q13 ranged from 26 to 37%. Using whole exome sequencing (WES) methodology, somatic *MEN1* mutation was found in some 35% of parathyroid benign tumors, comparable to results using conventional Sanger DNA sequencing [36, 37]. As mentioned above, HPT is the most penetrant feature of MEN1 and is usually the initial manifestation. As a result, true MEN1 families may sometimes be initially mis-assigned a clinical diagnosis of familial isolated hyperparathyroidism (FIHP) if only younger affected members are considered at the time that the family is ascertained (see **Figure 1**).

Mutation of the *MEN1* gene is only rarely associated with parathyroid carcinoma. The occurrence of parathyroid carcinoma in the context of familial MEN1 is extremely uncommon. Fewer than 20 patients with HPT due to parathyroid cancer in the context of the MEN1 syndrome have been reported [38]. LOH analysis of parathyroid tumor-extracted DNA has shown that DNA loss at the location of the *MEN1* gene on chromosome 11q, though frequently seen in benign parathyroid tumors, is quite uncommon in parathyroid carcinomas [39]. Recent studies that use next-generation WES of tumor-derived DNA to profile parathyroid cancers did not report any somatic mutations in *MEN1* [40, 41].
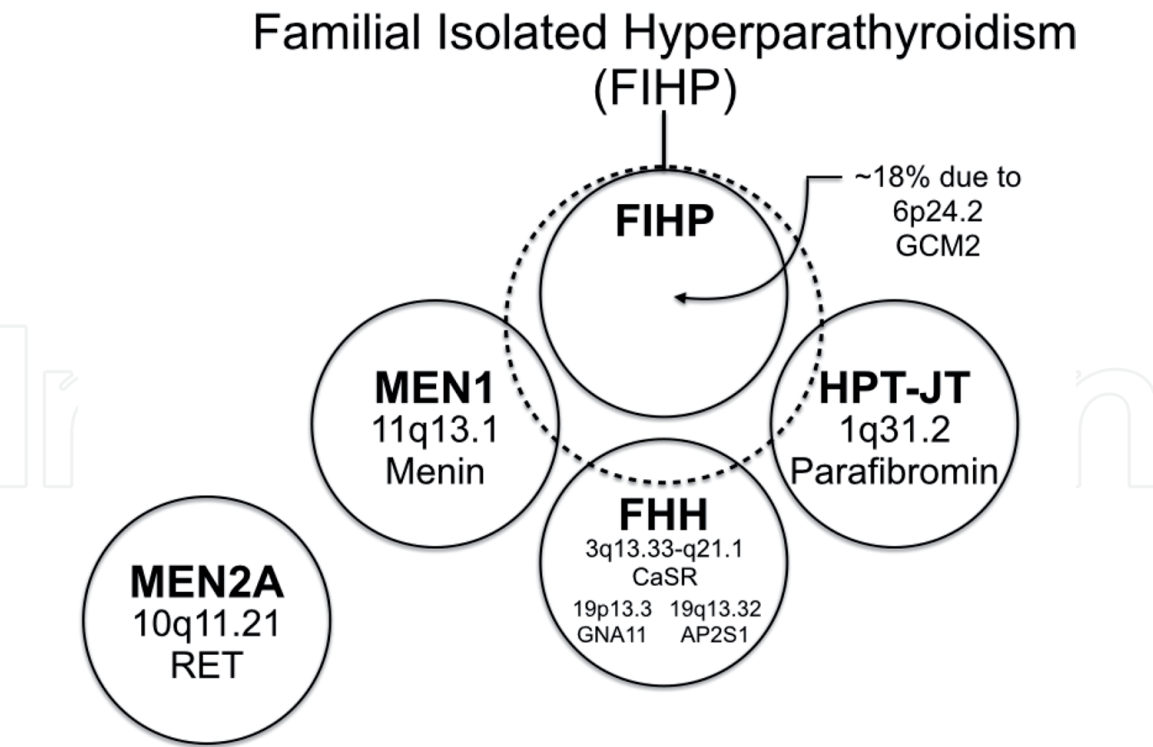
Familial Isolated Hyperparathyroidism (FIHP)

Figure 1.
The relationship among familial forms of hyperparathyroidism that may present as familial isolated hyperparathyroidism (FIHP) as a Venn diagram. The dashed circle represents the set of patients that can present with a provisional diagnosis of FIHP at the time of initial ascertainment. This includes patients with FIHP who have been evaluated for, but lack findings diagnostic of, MEN1, FHH and HPT-JT (nonsyndromic FIHP; in a solid circle). Approximately 18% of nonsyndromic FIHP kindreds harbor germline gain-of-function mutations in GCM2 (see text), whereas the remainder have currently unknown genetic etiologies. Subsets of patients with incomplete expression of MEN1, FHH and HPT-JT (the total set of patients in each syndrome represented by a solid circle) can also present with the FIHP phenotype (and thus overlap with the dashed circle). The distinction between the nonsyndromic FIHP category and the syndromic categories arbitrarily depends on the thoroughness of evaluation and the sensitivity of diagnostic tests used to detect the syndrome that can include germline gene mutational testing. MEN2A is a familial form of hyperparathyroidism that seldom if ever presents as FIHP. Within each circle representing a defined syndrome are included the genetic locus (or loci in the case of FHH; see text) of the syndromic trait and the associated gene product. The causative gene for HPT-JT encoding parafibromin is CDC73, formerly called HRPT2. The relationship among the patient sets illustrated as circles in this diagram is intended to be qualitative and neither the area of each circle nor the area of overlap between circles has any quantitative significance.

7. The hyperparathyroidism-jaw tumor syndrome (HPT-JT)

HPT-JT is a familial syndrome with variable and incomplete penetrance transmitted in an autosomal dominant fashion. The key clinical features of HPT-JT include HPT, jaw tumors (fibro-osseous tumors involving the maxilla and/or mandible, formally classified as cemento-ossifying fibromas [42], and distinct from so called "brown" tumors sometimes associated with HPT), renal cysts or tumors and uterine tumors in women [43–45]. HPT is the most penetrant feature of HPT-JT and is usually the presenting manifestation. In contrast to MEN1, parathyroid cancer is frequent in HPT-JT, affecting some 20% or more of those with HPT [43–46].

In the majority of HPT-JT kindreds, a germline loss-of-function mutation of the *CDC73* gene (formerly called *HRPT2*) can be identified [19, 47]. The majority of such *CDC73* mutations are predicted to inactivate gene function via frameshift or nonsense mutation, and only a minority of the mutations are missense [48]. Patients and kindreds with partial or complete deletion of the *CDC73* gene in the germline have also been described [49–52]. The *CDC73* gene encodes a 531-residue protein named parafibromin [47]. Because germline mutation predicted to cause loss-of-function of the *CDC73* gene predisposes to the neoplastic expressions

of HPT-JT, parafibromin is considered to be a tumor suppressor protein. Mixed epithelial tumor of the kidney (MEST), a rare type of renal tumor (formerly classified as cystic hamartoma of the renal pelvis, leiomyomatous renal hamartoma, or adult type mesoblastic nephroma), has been associated with HPT-JT and appears to correlate with a specific *CDC73* genotype, namely the Met1ILe missense mutation replacing the initiator methionine of parafibromin with isoleucine [47, 53, 54]. Somatic mutation of the *CDC73* tumor suppressor gene is uncommon in sporadic parathyroid adenomas [55]. In contrast to the results of analyses in benign parathyroid tumors, mutations of *CDC73* are quite frequently seen in apparently sporadic cases of parathyroid cancer [56–58]. Interestingly, recurrent somatic mutations in *CDC73* have been documented by exome sequence analysis of tumor DNA from parathyroid cancers [40, 41]. Selective amplification of the mutant copy of *CDC73* has been demonstrated in a subset of parathyroid carcinomas [40]. Approximately 25% of cases of seemingly sporadic parathyroid carcinoma may possess germline loss-of-function alterations in *CDC73*, suggesting that such patients may in fact have previously unrecognized, or *formes frustes* of, HPT-JT [19, 57, 58]. A minority of patients and families classified as FIHP can be shown to carry *CDC73* mutation in the germline, suggesting that this inherited disorder may in some cases be phenocopied by incompletely penetrant HPT-JT (see below and **Figure 1**). Approximately 20% of genetically confirmed or obligate *CDC73* mutation-positive family members lack HPT, fibro-osseous jaw tumors, or other manifestations of HPT-JT when their kindred is initially ascertained. Because the penetrance of the manifestations of HPT-JT increases with age among *CDC73* mutation carriers, lifelong surveillance of initially asymptomatic carriers is recommended [59].

8. Multiple endocrine neoplasia type 4 (MEN4)

MEN4 is a syndrome originally described by Pellegata and coworkers in a multigenerational family with features resembling MEN1, including a proband with a growth hormone-secreting pituitary adenoma and HPT, but lacking germline *MEN1* mutation [60, 61]. A germline heterozygous truncation mutation in *CDKN1B* was identified in the proband and several members of this kindred [60]. *CDKN1B* encodes the cyclin dependent-kinase inhibitor p27 (Kip1). Attention to the *CDKN1B* locus was a consequence of a previous genetic analysis of rats with the MenX phenotype, a recessively inherited condition caused by a frameshift mutation in *Cdkn1b* [60, 62]. The MenX phenotype in rats was manifest by the development of bilateral pheochromocytomas, paragangliomas, parathyroid adenomas and thyroid C cell hyperplasia [60, 62]. In the study by Pellegata et al., the proband was the only member of the MEN4/MENX kindred described who manifested HPT [60].

Following the original report by Pellegata et al. [60], several groups have investigated a possible role for *CDKN1B* mutation in parathyroid tumorigenesis. None of the earlier reports of *MEN1* mutation-negative families harboring germline mutation in *CDKN1B*, and expressing MEN1-like tumors and thus classified as MEN4, had included families with more than one member with HPT proven to track with the *CDKN1B* mutation [60, 63–71], apart from the demonstration of HPT linked to *CDKN1B* mutation in monozygotic twins [64]. That was true until a more recent report by Frederiksen et al. describing a large Danish family in which HPT occurred in 13 members, spanning two generations, who carried a germline frameshift *CDKN1B* mutation [72].

Recent evidence supports the characterization of *CDKN1B* as a susceptibility gene for the development of primary parathyroid tumors [69, 72, 73]. This evidence validates the inclusion of germline *CDKN1B* mutation in the differential diagnosis

of familial HPT, particularly in the evaluation of germline *MEN1* mutation-negative families who yet have MEN1-like features. The strongest justification for this follows from consideration of the Danish kindred in which 13 unique family members manifest HPT linked to germline inactivating mutation of *CDKN1B*, described by Frederiksen and co-workers [72].

9. Familial isolated hyperparathyroidism (FIHP)

By definition, FIHP is a non-syndromic category of familial HPT describing families that contain two or more members with HPT but which lack the specific features of MEN1, MEN2A, HPT-JT or FHH (**Figure 1**) [74]. FIHP is genetically heterogeneous and is a diagnosis of exclusion. While at the time of initial ascertainment germline mutation of *MEN1*, *CDC73*, or *CASR* may account for a fraction of kindreds with the FIHP phenotype [20, 34, 75–77], the majority of FIHP families lack mutations in these established HPT-susceptibility genes (**Figure 1**) [20, 75, 78].

Missense variants in GCM2, a transcription factor homologous to the Drosophila "glial cells missing" (gcm) gene and required for parathyroid gland development, were recently described in the germline DNA of eight unrelated families with FIHP [21]. Previous studies showed that germline dominant-negative and loss-of-function mutations in GCM2 were associated with autosomal dominant and autosomal recessive familial isolated hypoparathyroidism, respectively [79, 80]. The two rare germline *GCM2* genetic variants associated with FIHP act as gain-of-function mutations [21]. These missense mutations map to the C-terminal conserved inhibitory domain (CCID) of GCM2 and increase its transcriptional activity when measured *in vitro*, suggesting that *GCM2* in the context of FIHP is a parathyroid proto-oncogene. It has been estimated that approximately 18% of FIHP families harbor germline activating GCM2 mutations [21], leaving ~80% of FIHP families without a currently-identified genetic etiology [74]. Other clinical investigators have identified rare germline *GCM2* variants in a subset of FIHP kindreds [81]. Activating *GCM2* variants mapping to the CCID region have been found among patients with sporadic parathyroid tumors in low frequency and appear to be of low penetrance [82].

10. Familial hypocalciuric hypercalcemia (FHH)

FHH is a condition of PTH-dependent hypercalcemia, often resembling HPT, that is clinically benign and genetically heterogeneous (**Table 1**) [22]. Following partial or subtotal parathyroidectomy, affected patients from FHH kindreds almost always remain hypercalcemic. FHH is transmitted in an autosomal dominant fashion and usually causes mild hypercalcemia with relative hypocalciuria. The hypercalcemia seen in FHH is highly penetrant across all ages, including in infants [22, 83]. The majority of cases of FHH result from heterozygous germline inactivating mutation of the *CASR* gene on the long arm of chromosome 3 that encodes the calcium-sensing receptor [10, 84], and is classified as type 1 FHH (FHH1). Neonatal severe hyperparathyroidism (NSHPT), a rare autosomal recessive disorder typically presenting with severe hypercalcemia occurring in the first 6 months of life, most often results from the compound heterozygous or homozygous inheritance of two loss-of-function mutant *CASR* alleles [85]. Rather than the cellular monoclonality that would be expected in true parathyroid tumors, molecular genetic analysis of the hyperfunctioning parathyroid glands removed from a patient with NSHPT

demonstrated generalized polyclonal hyperplasia, underscoring the non-neoplastic nature of the abnormal parathyroid glands associated with *CASR* inactivating mutation [86].

Loss of surface expression of the CASR protein has been documented in parathyroid adenomas and may contribute to the altered calcium set point and impaired calcium-mediated negative feedback on the release of PTH typical of such adenomas. Decreased *CASR* mRNA expression, but not LOH at the *CASR* locus, has been documented in parathyroid adenomas [87]. In sporadic parathyroid tumors studied to date, somatic inactivation of the *CASR* gene has not been reported [88, 89].

Type 2 FHH (FHH2) resulting from germline loss-of-function mutation of *GNA11*, encoding the G protein α11 subunit [90, 91], and type 3 FHH (FHH3) resulting from germline inactivating mutation in *AP2S1*, the gene that encodes an adaptor protein involved in endocytosis mediated by clathrin [92–95], have also been described. In studies of sporadic parathyroid tumors, somatic inactivating mutations of *GNA11* and *AP2S1* have so far not been reported.

11. Multiple endocrine neoplasia type 2A (MEN2A)

MEN2A is a familial cancer syndrome characterized by a predisposition to the development of medullary thyroid cancer (MTC), pheochromocytoma (typically benign and often bilateral), and primary HPT. In the context of MEN2A, HPT is usually mild and resembles sporadic HPT. HPT in MEN2A is almost always results from benign parathyroid disease. MEN2A is an autosomal dominant disorder that results from germline gain-of-function mutation in the *RET* proto-oncogene at chromosomal location 10q11. *RET* encodes a receptor tyrosine kinase that binds the ligand glial derived neurotrophic factor, together with a glycosylphosphatidylinositol-anchored protein co-receptor Gfra1 [96].

Germline oncogenic mutations of *RET* are associated with three distinct familial endocrine neoplasia syndromes, all associated with MTC: MEN2A, multiple endocrine neoplasia type 2B (MEN2B), and familial medullary thyroid cancer (FMTC). The disease spectrum of typical MEN2B or FMTC does not include parathyroid tumors and HPT. Genotype–phenotype correlations based on particular *RET* mutations are apparent and account for the distinct patterns of disease. Some 95% of MEN2A cases are due to the presence in the germline of nonsynonymous variants affecting the RET receptor's extracellular cysteine-rich domain, namely missense mutations of *RET* codons 609, 611, 618, 620, or 634 [97]. In fact, germline missense alteration of RET residue cysteine-634 accounts for approximately 85% of cases of MEN2A [98].

12. Parathyroid tumorigenesis involving the CCND1 oncogene

The discovery of the *CCND1* (or *PRAD1*, for parathyroid adenomatosis 1) oncogene resulted from the analysis of several large, non-familial, parathyroid adenomas that harbored DNA re-arrangements that involved the PTH gene locus [99–101]. A breakpoint resulting from the pericentromeric inversion of chromosome 11 DNA was identified just upstream of the *CCND1/PRAD1* oncogene [101]. The inversion positioned the PTH gene regulatory region, that is normally located on the short arm of chromosome 11, just upstream of the *CCND1/PRAD1* proto-oncogene located on 11q [99–101]. The product encoded by the proto-oncogene

was subsequently recognized by DNA sequence analysis to be a member of the cyclin protein family [101]. The gene was therefore re-named cyclin D1 (*CCND1*). Overexpression of *CCND1* in the parathyroid cells of transgenic mice induces cell proliferation and gives rise to the metabolic abnormalities that characterize HPT in humans [102].

While activating CCND1 missense mutations have not been observed in sporadic parathyroid tumors [103], overexpression of *CCND1* has been demonstrated in 20–40% of sporadic benign parathyroid tumors and in an even larger percentage of parathyroid carcinomas [104–107]. In parathyroid carcinoma, no somatic chromosomal rearrangements on chromosome 11 involving *CCND1* have been reported. Neither germline activating missense mutations of *CCND1* nor chromosomal translocations or rearrangements involving the *CCND1* locus have been reported in any familial form of HPT.

13. Other genes involved in parathyroid tumorigenesis

Recurrent mutations in a subset of genes likely relevant to parathyroid tumorigenesis have been identified by WES analysis of DNA derived from sporadic parathyroid tumors. Eight out of 193 sporadic parathyroid tumors analyzed by WES demonstrated the Y641N missense mutation in the *EZH2* gene on chromosome 7 that encodes the enhancer of zeste 2 polycomb repressive complex 2 subunit [36]. Analysis by WES of 22 parathyroid tumors derived from a Chinese patient population identified a distinct somatic missense mutation, Y646N, in *EZH2* [108]. Acquired mutations of Y641 and Y646 in *EZH2* were described previously in lymphoid malignancy [109, 110]. Molecular genetic profiling of 80 sporadic parathyroid neoplasms by separate investigators failed to uncover any pathogenic *EZH2* mutations however, suggesting acquired *EZH2* mutation may be uncommon in parathyroid tumors [111]. In the context of lymphoma, *EZH2* is thought to function as a proto-oncogene [109]. To date, no transgenic mouse models restricting *EZH2* mutation or overexpression to parathyroid cells have been reported.

Soong and Arnold used WES analysis of DNA extracted from 19 parathyroid adenomas and matching germline DNA to identify somatic mutations in *ZFX*, a putative parathyroid proto-oncogene and member of the Krüppel associated box domain-containing family of zinc finger protein transcription factors [112]. Their observations in the discovery cohort were confirmed by direct sequencing of tumor DNA from an additional validation set comprised of 111 parathyroid adenomas [112]. The mutant *ZFX* alleles detected in parathyroid tumors likely act as oncogenes [113]. Such somatically acquired ZFX mutations in parathyroid tumors may be uncommon, however, since an independent mutational analysis of 23 sporadic parathyroid carcinomas and 57 adenomas failed to identify any pathogenic *ZFX* variants [111]. The development of a transgenic mouse model and/or better characterization of the functional properties of the mutant ZFX protein may clarify the potential significance of *ZFX* as a parathyroid proto-oncogene.

WES analysis of 22 blood-sporadic parathyroid adenoma tumor pairs from a Chinese patient cohort identified recurrent mutations of *ASXL3* [108]. ASXL3 belongs to a family of vertebrate Additional sex combs (Asx)-like proteins that may function as regulators of transcription. It remains unclear if the somatic missense *ASXL3* mutations identified in the parathyroid adenomas, mutations that affected highly conserved residues, would result in gain- or loss of ASXL3 function [108]. Further studies will be required to confirm this initial observation and to clarify the mechanism by which *ASXL3* mutation might drive parathyroid tumor development.

14. Conclusions

While inherited forms of HPT represent only a small fraction of cases (<5%), study of the molecular pathophysiology of these uncommon familial syndromes has yielded substantial insight into the genetic etiology of both sporadic and familial parathyroid disease and resulted in the identification of genes such as *MEN1, CDC73, CASR, GNA11, AP2S1, CDKN1B, CCND1*, and *GCM2*. It is highly likely that the mutational gain- or loss-of-function of other, yet unrecognized, genes is able to drive parathyroid neoplasia. For example, the risk in the majority of FIHP kindreds predisposing to the development of parathyroid tumors seems to result from the germline mutation of genes not presently recognized as having a role in parathyroid disease. This follows from the observation that nearly 70% of families initially considered as FIHP in multiple studies that examined for germline *MEN1, CASR* and *CDC73/HRPT2* gene mutation, had no recognized syndromic etiology (**Figure 1**) [20, 75–77]. From among those FIHP kindreds who are *MEN1, CASR* and *CDC73* mutation-negative, only about 20% are estimated to harbor germline activating mutations in the *GCM2* proto-oncogene [21], which leaves nearly 80% of FIHP kindreds with a currently-undefined genetic basis for their disease (**Figure 1**).

The existence of currently unidentified parathyroid tumor suppressors and oncogenes is also suggested by analysis of parathyroid tumors using techniques such as comparative genomic hybridization (CGH) to identify specific chromosomal regions harboring loss or gain of DNA. Several investigators have documented recurrent loss of DNA at the 1p, 6q, 9p, and 13q chromosomal loci in parathyroid tumors, indicating the potential presence there of novel parathyroid tumor suppressor genes [114–117]. The potential presence of novel oncogenes at chromosomal loci 9q, 16p, 19p, and Xq is suggested by results demonstrating specific chromosomal gain at these loci in benign or malignant parathyroid tumors [114, 116–118].

Next-generation sequencing analysis including WES of parathyroid neoplasms is an auspicious approach for the identification of novel acquired and germline gene variations that predispose to the development of HPT and parathyroid neoplasia. The apparent validation of this line of investigation by the identification of *EXH2* [36], *ZFX* [112], and potentially *ASXL3* [108], as candidate driver genes for parathyroid neoplasia was previously discussed. WES analysis of parathyroid cancer-derived DNA has similarly underscored the possible significance of recurrent somatic and germline inactivating mutations of *PRUNE2* in the etiology of parathyroid malignancy [40]. The comprehensive quality and great sensitivity of WES and related next-generation sequencing methodologies should further advance our insight into the genetic basis and endocrine pathophysiology of inherited and sporadic parathyroid neoplasia in the decades ahead.

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Conflict of interest

The author declares no conflict of interest.

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