Diagnosis, management, histology and genetics of sporadic primary hyperparathyroidism: old knowledge with new tricks

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Abstract

Primary hyperparathyroidism (pHPT) is a common endocrinopathy resulting from inappropriately high PTH secretion. It usually results from the presence of a single gland adenoma, multiple gland hyperplasia or rarely parathyroid carcinoma. All these conditions require different management, and it is important to be able to differentiate the underlined pathology, in order for the clinicians to provide the best therapeutic approach. Elucidation of the genetic background of each of these clinical entities would be of great interest. However, the molecular factors that control parathyroid tumorigenesis are poorly understood. There are data implicating the existence of specific genetic pathways involved in the emergence of parathyroid tumorigenesis. The main focus of the present study is to present the current optimal diagnostic and management protocols for pHPT as well as to review the literature regarding all molecular and genetic pathways that are to be involved in the pathophysiology of sporadic pHPT.

Introduction

Hyperparathyroidism is characterized by hypersecretion of parathyroid hormone (PTH) from the parathyroid glands. According to the cause of hypersecretion, hyperparathyroidism can be classified as primary, secondary or tertiary. Primary hyperparathyroidism (pHPT) is a common endocrinopathy resulting from inappropriately high PTH secretion from one or more enlarged parathyroid glands (1, 2). Secondary hyperparathyroidism usually results from low vitamin D levels or renal failure, while the tertiary form of the disease occurs when there is autonomous hypersecretion from one or more affected glands, despite resolution of secondary hyperparathyroidism (2).

The diagnosis may extend from normocalcemia accompanied by elevated PTH levels to hypercalcemia accompanied by elevated or inappropriately normal PTH levels. The incidence of pHPT is estimated to be 3:1000 in the general population and most cases are sporadic (3). However, 5–10% of the patients may be associated with syndromic and hereditary disease, like multiple endocrine neoplasia type 1 (MEN1), type 2A (MEN2A), type 4 (MEN4) and hyperparathyroidism jaw-tumor syndrome (HPT-JS) or the non-syndromic familial form of the disease, the familial isolated primary hyperparathyroidism (FIHP) (2, 4). Almost 90% of the patients are found to have sporadic, non-familial and non-syndromic disease (4). Sporadic
pHPT is usually caused by a single gland adenoma (85% of patients), but may also be caused by hyperplasia of all four glands (10%), double adenomas (2–5%) or rarely parathyroid carcinomas (<1%) (1, 2). The wide spectrum of disease presentation is very important as different management strategies are in demand.

There are data implicating specific genetic pathways in the emergence of adenomas, hyperplasias and carcinomas (5, 6), although it seems that epigenetic changes could also participate actively in parathyroid tumorigenesis (7, 8). The main focus of the present study is to present the current optimal diagnostic and management protocols for pHPT as well as to review the literature regarding all molecular and genetic pathways that are involved in the pathophysiology of sporadic pHPT.

**Diagnosis of pHPT**

The simultaneous presence of persistent hypercalcemia and elevated or inappropriately normal PTH levels, makes the diagnosis of pHPT likely (9, 10, 11), but not definite and alternative benign states such as familial hypocalciuric hypercalcemia (FHH) should be excluded (12). FHH is an autosomal-dominant disorder resembling pHPT, which is not cured by surgery (12). The presentation of both disorders may be confusing and the establishment of the diagnosis requires a careful and detailed workup.

Although the clinical presentation of patients with pHPT is heterogeneous and the associated symptoms may overlap with those of aging and disease (10), usually patients with symptomatic pHPT have overt signs and symptoms; however, the definition of symptomatic disease is still evolving. Patients with asymptomatic pHPT have no disease-specific symptoms but the diagnosis is always biochemical and well stated in the latest published guidelines on the diagnosis and management of the disease (10, 11, 13).

Another aspect of the disease is normocalcemic pHPT, which is an abnormality of the relationship between calcium and PTH. Normocalcemic pHPT presents with calcium levels within the normal range and the diagnosis could be revealed, with the use of calcium loading test: When an oral (or IV) load of 1 g elemental calcium or calcium gluconate infusion is given, serum ionized calcium concentration increases to supranormal values, and in normal individuals, a PTH suppression is expected (14). However, in patients with normocalcemic pHPT, only a minimal reduction in serum PTH concentration is seen (14). The reactivity of PTH to calcium loading could be used to establish the diagnosis of normocalcemic pHPT (14, 15, 16, 17, 18, 19, 20), as PTH and calcium levels may become inapproporiate only after the calcium load.

It is of great importance to adjust the total serum calcium levels not only for albuminemia but also for serum protein levels, as hyper- or hypo-proteinemia could lead to false diagnosis (9). If the adjusted total serum calcium level is normal, but PTH levels are elevated, then the ionized calcium levels could be measured, as pHPT could present with normal total but elevated ionized calcium levels (9, 11). The ionized calcium measurement should be performed in a pH-stable environment, as acidosis or alkalosis could cause respectively elevated or suppressed levels of ionized calcium (21). However, this measurement is of high cost and not widely available (11).

For the diagnosis of pHPT, other states that mimic pHPT should be excluded, such as FHH or medication intake (hydrochlorothiazide or lithium) (11). For the exclusion of FHH, calcium to creatinine clearance ratio (CaCrCR) is used and if the ratio is greater than 0.02, then the presence of FHH is unlikely, provided that the patient has normal renal function, there is no severe calcium or vitamin D deficiency and the patient is not under treatment with loop diuretics (11). In the presence of a CaCrCR <0.02, the CASR gene analysis may be helpful in establishing the diagnosis of FHH (22). However, it should be taken into account that there is high risk of diagnostic confusion between FHH and pHPT, as CaCrCR can greatly overlap between both hypercalcemic states (12). In addition, there are other genes involved in FHH, defining three different FHH subtypes (12). FHH type 1 is due to a mutation of the CASR gene, while FHH type 2 and 3 are caused by mutations of GNA11 and AP2S2 genes respectively (12). Moreover, another clinical entity that may raise diagnostic dilemmas has been recognized, ‘The Genetically Negative FHH’, which involves patients with an FHH phenotype (hypercalcemia, normal or slightly elevated PTH), no genetic abnormality of CASR, GNA11 and AP2S1 genes, at least one family member with the same phenotype and/or failure of surgical removal of parathyroid gland(s) to correct hypercalcemia (12).

Secondary causes for PTH elevation must be meticulously excluded as they mimic normocalcemic pHPT (9, 11, 23), like malabsorption syndromes, hypercalcioria and certain medications such as bisphosphonates, denosumab and loop diuretics. The presence of pHPT in children and young adults may be associated with the hereditary/syndromic forms of pHPT; however, analyzing the hereditary forms of pHPT is beyond the scope of the present review.
Localization of the disease

Although the diagnosis of pHPT is biochemical, the localization-imaging studies may reveal the gland or glands that are affected. Parathyroid imaging is not a diagnostic procedure and is advised only if parathyroidectomy (PTx) is planned (9). Once the PTx is decided, localization studies are the next step in order to identify the affected gland(s). The most common imaging techniques are $^{99m}$Tc-sestamibi scintigraphy, ultrasound and computed tomography (CT) (24). Parathyroid adenomas or multiple gland disease are not always identified, and thus, parathyroid imaging studies can be negative, but this should not preclude PTx, in specialized and experienced endocrine surgeons’ hands (11).

$^{99m}$Tc-sestamibi scintigraphy

This imaging technique is based on the preferential uptake of $^{99m}$Tc-sestamibi by the mitochondria-rich areas in parathyroid adenomas and hyperplasias (11). Normally, $^{99m}$Tc-sestamibi uptake is also observed in the thyroid, salivary glands, thymus, mammary gland during lactation, liver and bone marrow. This technique is sensitive (90%) and accurate (97.2%) for pHPT. There are two different options for $^{99m}$Tc-sestamibi technique, the single isotope washout scintigraphy and the two isotopes-subtraction scintigraphy ($^{99m}$Tc-sestamibi and $^{123}$I). In the single isotope technique, delayed washout is observed in well-defined areas indicating the parathyroid hyperfunctioning lesion. In the two-isotope technique, there is uptake of $^{99m}$Tc-sestamibi and $^{123}$I by the thyroid and uptake of $^{99m}$Tc-sestamibi only by the parathyroids. The subtraction images may reveal the parathyroid adenoma or multiple gland disease. The addition of single-photon emission tomography (SPECT) alone or in combination with low-dose CT usually improves the localization of the parathyroid lesion (11).

Magnetic resonance imaging (MRI)

MRI is used less commonly due to its lower sensitivity (50%); however, it is the imaging of choice for pregnant patients. The adenomas appear as a soft tissue mass with high signal intensity on T2-weighted frames, but low to moderate in T1-weighted frames (11). The signal intensity of the adenoma is enhanced after gadolinium injection on T1-weighted frames, compared to normal thyroid tissue. Lymph nodes may have the same appearance (11).

Computed tomography

CT is useful in localizing ectopic mediastinal parathyroid glands with a sensitivity of approximately 46–87%. Another technique that is being used recently with high sensitivity is the 4D-CT, with time being the fourth dimension. CT allows rapid assessment of the glands but has higher cost, exposure to radiation and requires iodinated contrast agents (11).

$^{11}$C-Methionine PET/CT scintigraphy

This imaging method is not widely available and is rarely used due to its high cost. It is based on the uptake and incorporation of the amino acid methionine in the PTH molecule during its synthesis (11).

Selective venous sampling with PTH measurements

This technique is mainly used in cases of unsuccessful surgery or reoperations. It requires an experienced angiographer, and it is expensive but has a high sensitivity, almost 75% when comparing with $^{99m}$Tc-sestamibi-SPECT (30%). When all non-invasive techniques have failed in revealing the lesion, then selective venous sampling is the imaging of choice in reoperation cases (11).

Ultrasound

Normal parathyroid glands are usually not detected by ultrasound. Parathyroid adenomas are usually seen as round or oval, hypoechoic structures, contrasting the hyperechogenic thyroid tissue (11). Large parathyroid adenomas may involve calcifications and cysts (11). Ultrasound is most useful in identifying adenomas close to the thyroid gland (11). Ultrasound may be useful as an additional study to confirm the localization of a parathyroid adenomas identified by $^{99m}$Tc-sestamibi (11).

Management of pHPT

The only definitive treatment for pHPT is PTx. Patients with symptomatic pHPT should be advised to undergo surgery, unless serious contraindications or significant comorbidities exist. However, surgical treatment can also be offered to the asymptomatic patients who meet the guideline criteria (age <50, serum calcium ≥1.25 at the lumbar spine, femoral neck, the total hip, distal 1/3 radius or low-energy fracture, glomerular
Table 1  Evolution of guidelines criteria for surgical management of asymptomatic pHPT throughout the years 1990–2016.

<table>
<thead>
<tr>
<th>Year</th>
<th>Age</th>
<th>Calcium levels</th>
<th>Renal function</th>
<th>Urine calcium excretion</th>
<th>Osteoporosis</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>1990a</td>
<td>&lt;50</td>
<td>1–1.6 mg/dL the upper limit or life-threatening hypercalcemia</td>
<td>eGFR reduction &gt;30%</td>
<td>&gt;400 mg/dL</td>
<td>Z-Score &lt;−2.0 (forearm)</td>
<td>Kidney stones detected by abdominal radiograph</td>
</tr>
<tr>
<td>2002b</td>
<td>&lt;50</td>
<td>&gt;1 mg/dL the upper limit</td>
<td>eGFR reduction &gt;30%</td>
<td>&gt;400 mg/dL</td>
<td>T-Score &lt;−2.5 (any site)</td>
<td>Presence of nephrolithiasis or nephrocalcinosis by X-ray, ultrasound, or CT</td>
</tr>
<tr>
<td>2008c</td>
<td>&lt;50</td>
<td>&gt;1 mg/dL the upper limit</td>
<td>eGFR &lt;60 mL/min</td>
<td>24h urine for calcium not recommended</td>
<td>T-Score &lt;−2.5 (lumbar spine, total hip, femoral neck, or distal radius) and/or fragility fracture diagnosed by imaging</td>
<td>Presence of nephrolithiasis, nephrocalcinosis or increased stone formation risk</td>
</tr>
<tr>
<td>2014d</td>
<td>&lt;50</td>
<td>&gt;1 mg/dL the upper limit</td>
<td>eGFR &lt;60 mL/min</td>
<td>&gt;400 mg/dL</td>
<td>T-Score &lt;−2.5 (lumbar spine, femoral neck, total hip, or the 1/3 radius) for postmenopausal women or males &gt;50 years. A prevalent low-energy fracture, which requires a routine X-ray of the thoracic and lumbar spine (or vertebral fracture assessment by DXA)</td>
<td></td>
</tr>
<tr>
<td>2016e</td>
<td>&lt;50</td>
<td>&gt;1 mg/dL the upper limit</td>
<td>eGFR &lt;60 mL/min</td>
<td>&gt;400 mg/dL</td>
<td>T-Score &lt;−2.5 (lumbar spine, femoral neck, total hip, or the 1/3 radius) for postmenopausal women or males &gt;50 years. A prevalent low-energy fracture, which requires a routine X-ray of the thoracic and lumbar spine (or vertebral fracture assessment by DXA)</td>
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eGFR: estimated glomerular filtration rate.

filtration rate (GFR) of <60 mL/min, nephrocalcinosis, renal stones or high stone risk). Table 1 presents the latest guidelines in comparison with older ones for the management of asymptomatic pHPT. Data from cohort studies demonstrated reductions in the risk of all fractures and renal stone formation post-PTx (25, 26, 27, 28).

Cure rates in specialized endocrine surgeons hands exceed 95%, with complications rates as low as 1–3% (29). In patients with a single adenoma (85% of patients), surgery can be curative. In the 15% of patients with multiple gland disease, the recurrence rate is higher and requires a subtotal PTx. Parathyroid carcinoma is rarely (<1%) the cause of pHPT. If a patient is suspicious for carcinoma (tumor size >3 cm, palpable mass, and serum calcium >14 mg/dL) (30), en bloc resection of the ipsilateral thyroid lobe and any invaded tissues should be performed (29).

PTx benefits may also include cardiac and vascular function (31), neurologic and gastrointestinal health and most patients report a general clinical improvement (29). The complications of PTx are rare and may include failure to cure the disease by not finding the affected gland(s), persistence or recurrence of the disease, recurrent laryngeal nerve damage, damage in the normal parathyroid glands, bleeding or hematoma and infection. Patients should be advised to maintain unrestricted calcium intake and sufficient vitamin D levels before surgery in order to avoid hungry bone syndrome.

Successful preoperative localization of a parathyroid adenoma is a good indication for minimally invasive parathyroidectomy in non-syndromic patients with sporadic pHPT. The benefits of this technique are reduced operative time, outpatient surgery, reduced costs and fewer complications (29). As suggested by the latest guidelines, if preoperative imaging has not identified a parathyroid adenoma or hyperplasia or if familial disease is highly suspected, bilateral neck exploration PTx is preferred (29). A bilateral neck exploration and subtotal PTx is usually performed in syndromic patients or in patients with lithium-induced pHPT (11). The minimally invasive approach can be extended to a bilateral neck exploration if an adenoma cannot be found or if the patient has unsuspected multiglandular disease. Multiglandular pHPT may not be excluded before surgery and is seen in almost
one in ten patients with sporadic pHPT (29), and in these cases, bilateral neck exploration is mandatory.

Patients with asymptomatic pHPT who do not meet guidelines for surgery or are unable or unwilling to undergo PTx may be offered monitoring, with unrestricted calcium intake and maintenance of sufficient vitamin D levels. Antiresorptive treatment (bisphosphonates or hormone replacement therapy) should be considered in patients with osteoporosis or in the presence of fragility fractures in patients who are unable or unwilling to undergo PTx (11).

Another option is cinacalcet, a calcimimetic agent, which lowers serum calcium and PTH by increasing the sensitivity of the CaSR to extracellular calcium, thereby decreasing serum PTH and reducing the renal tubular reabsorption of calcium. With cinacalcet, serum calcium normalizes in 70–80% of patients with pHPT, but fails to normalize PTH in about 50% of patients. European Medicines Agency (EMA) and The Food and Drug Administration agency of the United States (FDA) have approved the use of cinacalcet for the treatment of secondary hyperparathyroidism in patients with chronic kidney disease (CKD) on dialysis, for treating hypercalcemia in patients with parathyroid carcinoma and for the treatment of severe hypercalcemia in patients with primary HPT who are unable to undergo parathyroidectomy. The main disadvantage of this cinacalcet is its high cost and a high incidence of adverse reactions, mainly from the gastrointestinal system. It is a useful option in symptomatic patients in whom the disease cannot be controlled by surgical intervention or in circumstances where surgery is contraindicated and in patients with unresectable parathyroid cancer (11). This drug also does not improve bone mineral density or lower biochemical markers of bone turnover (32). There are not sufficient data on its effects on hypercalcemic symptoms, renal stones or quality of life. Treatment with cinacalcet is responsible for urinary calcium excretion, either by reduction of tubular calcium reabsorption via the reduction of PTH level or via a direct effect on the calcium sensor receptor located in the upper thick ascending limb of the loop of Henle. However, recent studies reported that patients carrying the rs1042636 polymorphism of the calcium-sensing receptor gene respond more sensitively to cinacalcet and have a higher risk of calcium stone formation (33). Data with medical therapy currently is short term and insufficient to justify medical therapy as an alternative to surgery (11).

Histopathological features of parathyroid neoplasms

The histological distinction between the various forms of primary hyperparathyroidism is challenging as the differential diagnosis between a single adenoma and multiple gland disease is difficult. When in sporadic pHPT only one gland is affected, it usually bears only one adenoma. However, when multiple glands are involved, they may present a range of pathology from diffuse hyperplasia to asymmetric hyperplasia or multiple adenomas. Moreover, the histological diagnosis of parathyroid cancer can be challenging and in some cases diagnosis can only be done following revelation of distant metastases. However, there are some pathological features that are used for the histological classification of the tumors, and these features are being analyzed.

Primary adenoma

The incidence of parathyroid adenomas in sporadic pHPT is estimated to be 85–90% (2). They are usually single, while multiple affected glands account for 20% of the cases and carcinomas for <1% of cases (34). ‘Double’ or ‘multiple’ adenomas may at least in some cases alternatively represent multigland hyperplasia, presenting asymmetrically and sometimes asynchronously (34).

Most adenomas are composed of chief cells, a small percentage may be oxyphilic, large clear cell adenomas or rarely ‘water-clear’ adenomas or lipoadenomas (34, 35). Typical parathyroid adenomas are surrounded by a fibrous capsule; however, microadenomas (weighting <0.1 g) are usually non-encapsulated. A rim of normal parathyroid tissue is often present at the periphery of adenomas, although this feature may not be always present. The component cells may be arranged in cords, nests, sheets and follicles and may arrange around blood vessels. The round and densely stained nuclei are larger than those present in the non-neoplastic parathyroid tissue. The nuclei are also hyperchromatic and pleomorphic, the so-called ‘endocrine atypia’ (36).

The oxyphilic (or oncocytic) adenomas consist mainly of oxyphilic cells and are usually non-functional. They are usually soft, ellipsoid or lobulated with variable brownish colors. Microscopically, oxyphilic adenomas are composed of sheets, anastomosing cords and acinar arrangement of polygonal cells with abundant granular, brightly eosinophilic cytoplasm and centrally placed rounded nuclei (35).
The large clear cell (light chief cell) adenoma is another rare variant of adenoma, and they are comprised of polygonal cells with cytoplasmic vacuoles filled with glycogen. They exhibit positivity in Periodic Acid-Schiff (PAS) staining, with or without diastase digestion. The water-clear cell adenoma consists of nests and acini with clear cells containing abundant foamy, granular cytoplasm and mild nuclear pleomorphism. They are thought to be large clear cell adenomas with glycogen accumulation rather than true water-clear cells with membrane bound vesicles. Lipoadenomas have also been described and are characterized by nests and cords of chief cells with a few oxyphil cells intimately admixed with variable amount of mature adipose tissue and fibrous stroma (34). They may be non-functional or rarely may present with primary hyperparathyroidism (35).

It should also be mentioned that there are tumors with some features suggesting malignancy, but falling short of unequivocal histological diagnosis of malignancy, which are termed ‘atypical adenoma’. The usual histopathological features include fibrous bands, pronounced trabecular growth, remarkable mitotic activity (>1 mitosis per 10 high-power fields), tumor necrosis and incomplete invasion of the capsule. The significance of the remarkable mitotic activity is unclear. Their detection should prompt careful assessment, while searching for other features of malignancy (34).

**Primary hyperplasia: multiple gland disease**

Parathyroid hyperplasia usually affects 10–15% of sporadic cases with pHPT. It involves may be diffuse or asymmetrical and involve some glands (34, 37). Asynchronous presentation may also occur (34). The glands are enlarged, hypercellular and functioning with decreased or absent intracellular fat, as indicated by fat staining (38). The glands may demonstrate diffuse proliferation of parenchymal cells, with little or no extracellular fat or nodular development consisting mainly by chief cells, although foci of oncocyes and clear cells may be admixed (37).

There are two major types of histological hyperplastic proliferation patterns. One is the chief cell hyperplasia pattern and the other is the clear cell hyperplasia pattern. The above variation implies that there are different molecular pathways that lead to the development of hyperplastic multiple gland disease. Primary chief cell hyperplasia may be observed predominantly in sporadic and also in hereditary forms of pHPT.

Hyperplasia occurs predominantly in chief cells in this histological subtype. Gland enlargement can by either symmetrical or asymmetrical occurring only in some glands. Of interest is the fact that although within a single gland the growth pattern is usually diffuse, in some cases, it presents with a nodular intraglandular pattern of growth with foci of normal parathyroid tissue. The latter is seen in hereditary disease, usually multiple endocrine neoplasia type 1, suggesting formation of multiple adenomas involving all glands rather than true diffuse hyperplasia in these patients.

Water cell hyperplasia is rare and is never associated to hereditary disease. In this entity all glands are diffusely pathologic (36).

**Parathyroid carcinoma**

Parathyroid carcinoma is a rare lesion whose histological diagnosis could be challenging. It may require the presence of distant metastasis in order to confirm the diagnosis. Histological findings could include those seen in atypical adenomas with some additional evidence of invasion. Abnormal mitotic activity appears to be a feature, but not pathognomonic of this entity. The World Health Organization suggests that the following criteria must be fulfilled in order to confirm the diagnosis: presence of vascular invasion (in the capsule or adjacent tissues), capsular invasion with extension to adjacent tissues and/or presence of metastases.

Recently, De Lellis mentioned that most carcinomas have a solid growth pattern with tumor cells arranged in diffuse masses, small nests or trabeculae, some tumors exhibiting spindle cell, follicular or papillary patterns (39, 40). There is no variation in nuclear size and shape, and this may be an indistinguishable feature from adenomas. Some tumors exhibit pleomorphism with coarse chromatin and macronucleoli, a feature that must be distinguished from the so-called endocrine atypia encountered in parathyroid adenomas and other benign endocrine tumors (39).

**Molecular pathways and genetic involvement in sporadic pHPT**

Tumorigenesis may involve multiple different mechanisms responsible for hyperplastic changes such as activation of oncogenes, inactivation of tumor suppressor genes, imbalance between growth factors and proteins involved in cell regulation and epigenetic alterations.
Pathways and genes involving cell cycle regulators

The cell cycle is a series of events that take place in the cell and lead to its division. Cyclins are proteins that are involved in the regulation of progression of cells through the cell cycle and activate cyclin-dependent kinases (CDKs) (43). The expression of cyclin genes regulates the activity of CDKs (43). Elevated levels of cyclin D1 (CCND1, PRAD1) have been shown to promote cell cycle progression. CCND1 gene has been found to be overexpressed in sporadic parathyroid adenomas and carcinomas (43, 44, 45). On the other hand, CDK inhibitors also regulate cellular proliferation and apoptosis, by inhibiting the complex cyclin-CDK (43). CDK inhibitor 1B (P27, CDKN1B) and CDK inhibitor 1A (P21) are members of the CDK inhibitor proteins. Studies have shown that sporadic parathyroid carcinomas have decreased P27 and P21 expression (43), while low-frequency germline and somatic mutations have also been reported in patients with adenomas (46, 47, 48). CCND1 gene has been found to be overexpressed in sporadic parathyroid adenomas and carcinomas (43, 44, 45) (Fig. 1).

Another inhibitor of cell cycle progression is retinoblastoma protein (RB). It works as a tumor suppressor factor. Allelic loss of the RB gene was found to be involved in parathyroid carcinomas (25). Proliferating cellular nuclear antigen (PCNA) has been studied and shown to interact with other proteins involved in DNA replication and repair, cell cycle control, chromatin remodeling/epigenetic inheritance, chromatin cohesion and transcription (49). PCNA acts as a cofactor for DNA polymerase D and interacts with CYCLIN D1 (49, 50). It was recently demonstrated that PCNA was considerably higher in adenomas, followed by primary hyperplasias, whereas PCNA in common adenoma and other hyperplastic changes were even lower than those in healthy parathyroid glands (51, 52).

Recent studies showed that about 40–45% of sporadic parathyroid tumors show MEN1 gene mutations. Sporadic parathyroid tumors harboring MEN1 gene somatic mutations frequently evidence LOH on chromosome region 11q13 (53, 54, 55). Targeted inactivation of MEN1 gene specifically to the parathyroid glands resulted in parathyroid neoplasia accompanied by hypercalcemic hyperparathyroidism (56). Loss of heterozygosity of chromosome 11q, the genomic location of the MEN1 gene, is the most frequent genomic aberration found.

Figure 1
Parathyroid tumorigenesis mechanisms via the cyclins pathway. CCND1 gene, encoding cyclin D1, is upregulated in parathyroid adenomas. MEN1 gene inactivation results in a reduction of P27 and inhibition of cyclin and CDK complexes, as well as a loss of control of cell cycle progression. Inactivating somatic and germline mutations of CDC73 are frequently identified in patients with parathyroid carcinoma. CDKN1B has been reported to be downregulated in adenomas compared to normal tissues.
in parathyroid adenomas (56). The product of MEN1 gene, called menin, can function as suppressor of transcription, because this protein is able to bind a family of transcription factors such as AP-1/Jun-Fos family, and it is also associated with a histone methyltransferase (HMT) complex leading to an increased expression of cyclin-dependent kinase inhibitors (CDKIs) and consequently suppressing cell growth (57, 58).

Latest data on sporadic pHPT have concluded on specific genes being upregulated or downregulated, mostly on sporadic adenomas (4). Non-familial pHPT patients (almost 90% of patients with pHPT) may carry somatic mutations in specific genes like MEN1, CCND1, RB, RIZ1, CTNN3 and LRP5 genes (4). However, sporadic forms of pHPT may also be associated with germline mutations involving MEN1, CDC73, CASR, CDKI or PTH genes (4). It is estimated that 10% of patients presenting with sporadic pHPT under the age of 45 years, carry a de novo germline mutation in MEN1, CDC73 or CASR genes (4, 59, 60). In addition, almost 5% of patients with pHPT presenting in the sixth to ninth decades of life with a single adenoma and without family history of parathyroid disease, MEN or other endocrine tumor syndromes, may carry germline mutations of the CDKI genes (usually involving CDKN1A (P21), CDKN2B (P15) or CDKN2C (P18)) (4, 61). A PTH nonsense mutation, Arg83Stop, has also been associated with hypercalcemia due to parathyroid adenoma and an undetectable PTH (62).

**Wnt/β-catenin signaling pathway and genetic alterations causing its dysfunction in pHPT**

Another important pathway that should be mentioned is the Wnt/β-catenin signaling pathway, which affects multiple cellular functions mainly through gene transcription. Dysregulation of this pathway with subsequent accumulation of β-catenin in the cell cytoplasm or nucleus has been implicated in carcinogenesis (48). When cells bind a Wnt-ligand, the protein binds to frizzled receptors, LRP5/6 co-receptors and a destruction complex (for example, APC, AXIN, GSK-3B, DVL). This binding causes Axin dephosphorylation and degradation and the production of the non-phosphorylated and active β-catenin. The latter binds to the LEF/TCF family of transcription factors and regulates gene transcription (48). Mutant proteins of the Wnt pathway have been associated with some forms of cancer. Stabilizing β-catenin mutations and resistance to cytoplasmic dephosphorylation and degradation, causes accumulation of non-phosphorylated active β-catenin and have been associated with parathyroid tumorigenesis and pHPT (48, 63). In addition, the absence of stabilizing mutations of beta-catenin encoded by CTNNB1 exon 3 has been reported in a large series of sporadic parathyroid adenomas (64).

Two low frequency β-catenin stabilizing mutations have been identified and reported in parathyroid adenomas (Ser37Ala (48, 63) and Ser33Cys (63, 65, 66)), but more studies are required to substantiate this genetic disorder. In parathyroid adenomas without a stabilizing β-catenin mutation, the accumulation of the protein may also be due to an aberrantly spliced internally truncated Wnt receptor LRP5 (LRP5Δ). This disabled receptor was found to be expressed in 86% of the investigated parathyroid adenomas causing pHPT (67). In some investigated parathyroid adenomas, the MYC oncogene was found to be overexpressed and regulated by β-catenin activity in a human parathyroid cell line, as was expression of the CCND1 oncogene (68, 69).

**Pathways involving growth factors and reported genetic predisposition to pHPT**

Growth factors can affect cellular growth, proliferation, tissue repairing and homeostasis and any imbalance could lead to tumorigenesis (43). Angiogenesis is a key process for the emergence and survival of proliferative lesions, as it involves the formation of new vessels from existing ones to ensure that oxygen and other necessary nutrients will be transferred to the tumor. Factors that are found to be overexpressed in pHPT include vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), transforming growth factor beta (TGFβ) and insulin growth factor-1 (IGF-1) (43). Genes affecting growth factor expression are found to be involved in sporadic pHPT.

VEGF is regulating angiogenesis in normal or pathological states. It is implicated in tumorigenesis and in parathyroid proliferating lesions, it has a proangiogenic effect (43). Basic FGF (FGF-2) is also found to be upregulated in parathyroid tumors (70). FGF-2 is involved both in cell cycle regulation as well as in angiogenesis, tissue growth and repair (43, 71). FGF-2 and VEGF act synergistically on angiogenesis in cultured epithelial cells (71, 72). TGFβ is found to affect multiple cellular processes, including angiogenesis, and it is increased in patients with pHPT (73). In low dosage, it acts in favor of cell proliferation, while in high dosage, it inhibits proliferation (43). IGF-1 is the effector for the action of growth hormone. It is also a mediator of parathormone anabolic effects on bones. It is found to stimulate cellular proliferation, and it is implicated in the growth regulation of parathyroid tumors (74).
Apoptotic pathways and genetic factors involved in pHPT

Apoptosis is a form of programmed cell death, which ensures the tissue homeostasis and the maintenance of certain number of cells (75). Mechanisms involved in the pathogenesis of pHPT also include an imbalance between apoptotic and anti-apoptotic factors, some of which are tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), Fas receptor, the BCL family, the mouse double minute 2 homolog (MDM2) and tumor protein 53 (P53) (43, 75).

TRAIL is a protein that induces caspase 8-dependent apoptosis, via death receptors DR4 and DR5 (75). It has been implicated in anticancer activity and cardiovascular disease prevention, lipid uptake and blood vessel homeostasis (75, 76, 77, 78, 79). However, TRAIL can have an opposite role in apoptosis when forming complexes with transmitters such as Fas-associated with a death domain (FADD) or caspase-8 (80, 81). TRAIL gene has been found to be overexpressed in parathyroid adenomas and hyperplasias compared to normal tissue (43).

FAS receptor is a death receptor causing apoptosis by forming the death-inducing signaling complex (DISC) (75). Parathyroid adenomas and hyperplasias demonstrated overexpression of the FAS gene compared to normal tissue. Hyperplasias showed the highest expression, whereas in adenomas, the expression of the FAS gene was increased compared to normal tissue, but it was lower than that in hyperplasias (41).

The BCL-2 is a family of proteins that regulates caspase activation and mitochondrial outer membrane permeabilization (43). The anti-apoptotic BCL-X(L) and BCL-W genes were higher in adenomas compared to hyperplasias (43). The expression of pro-apoptotic members of the BCL-2 family, like BIM and BOK, was decreased in hyperplasias (BCL-W genes were higher in adenomas compared to hyperplasias (43)). The anti-apoptotic BCL-X(L) and BCL-W genes were higher in adenomas compared to hyperplasias (43).

Mutations in the BIM gene may lead to resistance to apoptosis (43). MDM2 is a negative regulator of the tumor suppressor P53, acts as an oncogene, and it is found to be overexpressed in multiple tumor types like sarcomas and breast cancer (43). It seems that BCL-2 and MDM2 have no expression in parathyroid carcinoma, while they are expressed in adenomas and hyperplasias (41, 43). In addition, P53 was found to be overexpressed in parathyroid carcinomas, Fas and p53 expression is correlated in hyperplasia, while there is no relation between FAS, Ki-67 and PCNA (43, 82).

There are a few recent studies trying to set the genetic background of primary parathyroid hyperplasia vs adenoma via the differential gene expression profile (83, 84, 85, 86). Data from one study revealed more than 200 genes being differentially expressed among hyperplasia and adenoma (85). The most statistically significant ones being upregulated were the HOOK 1 protein, the thromboxane A2 receptor gene (TBXA2R) and the fragile histidine triad gene (FHIT) (85). The downregulated genes were the prostaglandin-d synthase gene (PTGDS) and EGFR (downregulated in hyperplasia) (85). All referred genes are listed in Table 2.

miRNA differential expression and parathyroid tumorigenesis

MicroRNAs (miRNA) are small non-coding, single-stranded RNAs, 19–25 nucleotides long, which exert regulatory functions such as regulation of gene expression through multiple mechanisms including decreased

### Table 2 Genes reported to be involved in sporadic pHPT.

<table>
<thead>
<tr>
<th>Genes</th>
<th>Adenoma</th>
<th>Multiple gland disease</th>
<th>Carcinoma</th>
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transformation, increased degradation of the target messenger RNA (mRNA) or both (8). Binding of miRNA to its target mRNA results in the repression of translation (8). miRNA genes may act as oncogenes or tumor suppressor genes (87). Differentially expressed miRNAs have been reported in parathyroid adenomas and carcinomas, compared to normal parathyroid tissue (88). Recent data suggest a specific expression pattern of miRNAs in parathyroid carcinoma (2, 89). Among the downregulated miRNAs, miR-296, miR-139, miR-126-5p, miR-26b and miR-30b are differentially expressed in parathyroid carcinomas (2, 82). However, there are limited data on miRNA expression profile in the so-called sporadic primary hyperplasia (2), compared to parathyroid adenoma or carcinoma. Rahbabi and coworkers suggested the existence of 22 uniquely expressed miRNA in parathyroid hyperplasia, which need to be validated in other cohorts as well (2).

Summary-clinical implications

Sporadic pHPT is a common clinical entity with many of its aspects needed to be elucidated. It would be of great interest to investigate further the genetic deregulation of parathyroid tumors, as it may provide new aspects in the earlier and accurate pre-operative diagnosis and management. The clarification of the molecular pathways affecting parathyroid tumorigenesis will elucidate the pathogenetic mechanisms and will probably suggest new therapeutic approaches, targeting in the repression of deregulation of the affected pathways.

Declaration of interest

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

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