Clinicopathological correlates of hyperparathyroidism

Kai Duan,1,2 Karen Gomez Hernandez,3,4 Ozgur Mete1,2,3

ABSTRACT

Hyperparathyroidism is a common endocrine disorder with potential complications on the skeletal, renal, neurocognitive and cardiovascular systems. While most cases (95%) occur sporadically, about 5% are associated with a hereditary syndrome: multiple endocrine neoplasia syndromes (MEN-1, MEN-2A, MEN-4), hyperparathyroidism-jaw tumour syndrome (HPT-JT), familial hypercalcemic hypercalcaemia (FH-H, FH-2, FH-3), familial hypercalcuria hypercalcaemia, neonatal severe hyperparathyroidism and isolated familial hyperparathyroidism. Recently, molecular mechanisms underlying possible tumour suppressor genes (MEN1, CDC73/HHRPT2, CDKits, APC, SFPRs, GSK3β, RASSF1A, HIC1, RIZ1, WT1, CaSR, GNA11, AP2S1) and proto-oncogenes (CCND1/PRAD1, RET, ZFX, CYNNB1, EZH2) have been uncovered in the pathogenesis of hyperparathyroidism. While bi-allelic inactivation of CDC73/HHRPT2 seems unique to parathyroid malignancy, aberrant activation of cyclin D1 and Wnt/β-catenin signalling has been reported in benign and malignant parathyroid tumours. Clinicopathological correlates of primary hyperparathyroidism include parathyroid adenoma (80–85%), hyperplasia (10–15%) and carcinoma (<1–5%). Secondary hyperparathyroidism generally presents with diffuse parathyroid hyperplasia, whereas tertiary hyperparathyroidism reflects the emergence of autonomous parathyroid hormone (PTH)-producing neoplasms occurring from secondary parathyroid hyperplasia. Surgical resection of abnormal parathyroid tissue remains the only curative treatment in primary hyperparathyroidism, and parathyroidectomy specimens are frequently encountered in this setting. Clinical and biochemical features, including intraoperative PTH levels, number, weight and size of the affected parathyroid gland(s), are crucial parameters to consider when rendering an accurate diagnosis of parathyroid proliferations. This review provides an update on the expanding knowledge of hyperparathyroidism and highlights the clinicopathological correlations of this prevalent disease.

INTRODUCTION

Hyperparathyroidism (HPT) is a common endocrine disorder characterised by oversecretion of parathyroid hormone (PTH), which may be autonomous (independent of serum calcium levels) or the result of a physiological stimulus.1-11 Our understanding of this disease has progressed substantially over the past decade, with advancements made in biochemical, radiological and molecular testing.13 12 13 Early detection is crucial to prevent complications from uncontrolled HPT, affecting the kidneys (ie, nephrolithiasis or nephrocalcinosis) and bones (ie, osteoporosis, osteitis fibrosa cystica).14 7 14-19 Furthermore, neurocognitive dysfunction and cardiovascular morbidity and mortality appear to be increased in patients with overt HPT, although the nature and reversibility of these effects remains less evident.14 15 20-25

HPT is generally divided into three types: primary (autonomous HPT), secondary (HPT that results from a chronic stimulus causing PTH secretion) and tertiary (emergence of autonomous HPT in refractory secondary HPT).1-9 Primary HPT is currently the most common cause of hypercalcemia, with an annual incidence ranging from 34 to 120 cases per 100 000.4 7 14-26-28 While most cases are sporadic (95%), about 5% present with a hereditary syndrome: multiple endocrine neoplasia syndromes (MEN-1, MEN-4, MEN-2A), hyperparathyroidism-jaw tumour syndrome (HPT-JT), familial hypercalcemic hypercalcaemia (FH-H, FH-2, FH-3), familial hypercalcuric hypercalcaemia, neonatal severe hyperparathyroidism and isolated familial HPT.19

Surgical resection of abnormal ‘hyperfunctioning’ parathyroid gland(s) remains the only curative treatment for primary HPT.1 4 7 20-23-27-29 Thus, parathyroidectomy specimens are frequently encountered in this setting.18-34 Clinicopathological correlates of primary HPT include parathyroid adenoma (80–85%), hyperplasia (10–15%) and carcinoma (<1–5%).4 7 18-33 41 41 52 62 Secondary HPT typically presents with diffuse parathyroid hyperplasia, whereas tertiary HPT reflects the emergence of autonomous PTH-producing adenoma or rarely carcinoma from a background of secondary hyperplasia.7 11 42 44 46 Accurate subtyping is important for treatment decision-making and requires a thorough integration of pathological features with clinical, biochemical, radiological and intraoperative findings.4 7 42 49-51 This review provides an update on the evolving knowledge of HPT and highlights the clinicopathological correlations of this important disease.

CLINICAL AND BIOCHEMICAL FEATURES

Clinically, sporadic primary HPT can present at any age, with an increased incidence in postmenopausal women.4 8 10 42 52 53 A wide spectrum of clinical manifestations can be encountered, depending on the duration and degree of PTH oversecretion and resultant hypercalcemia (tables 1 and 2).4 8 7 12-14 In the past, symptomatic disease was the most common presentation, prompting the ‘classic’ description of ‘stones’ (ie, nephrolithiasis), ‘bones’ (ie, osteitis fibrosa cystica) and ‘groans’ (ie, constipation, ileus).4 6 12 14 15 However, with the advent of routine serum calcium testing, most cases (70–80%) are now diagnosed incidentally, in the ‘asymptomatic’ form.12 14 The natural progression from asymptomatic to symptomatic disease remains unclear at this time.4 7 12 14 However, even its ‘asymptomatic’ form, HPT is associated with...
potential morbidities, including decreased trabecular/cortical bone density and clinically ‘silent’ nephrolithiasis/nephrocalcinosis, which can be detected radiographically.6 12 14 16–19 54–62 In light of these findings, all cases of incidentally detected hypercalcaemia merit additional investigations, including ‘intact’ serum PTH measurement, to clarify its aetiology (PTH dependent vs PTH independent).467 Other commonly described symptoms in HPT are associated with hypercalcaemia (ie, weakness, easy fatigability, anxiety, cognitive impairment).4 7 12 14 15 20 54

Gastrointestinal manifestations are almost never reported in the modern presentation of primary HPT.5 14 15 63 64 Biochemically, mild hypercalcaemia (usually within 0.25 mmol/L of normal range) is often the first finding in sporadic primary HPT.4 6 7 63 Typically, urinary calcium is normal or increased although it may be low in individuals with concomitant thiazide or lithium use.66 67 A persistently low calcium after thiazide or lithium discontinuation should raise the possibility of FHH. A normocalcemic variant of primary HPT.

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<th>Table 1</th>
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<tr>
<td>Syndrome</td>
<td>Primary hyperparathyroidism</td>
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<td><strong>Definition</strong></td>
<td>Autonomic PTH oversecretion from abnormal parathyroid gland(s)</td>
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<td><strong>Clinical manifestations</strong></td>
<td>Often asymptomatic; more symptomatic in malignant HPT</td>
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<tr>
<td><strong>Biochemistry</strong></td>
<td>Mild hypercalcaemia with elevated PTH levels; more severe findings in malignant HPT</td>
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<td><strong>Radiology</strong></td>
<td>Single-gland parathyroid disease (80–85%) or multigland disease (10–15%)</td>
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<tr>
<td><strong>Pathology</strong></td>
<td>Parathyroid adenoma (80–85%), hyperplasia (10–15%) and/or carcinoma (&lt;1–5%)</td>
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<tr>
<td><strong>Treatment</strong></td>
<td>Surgery</td>
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*Secondary causes of hyperparathyroidism include chronic kidney disease (most classic), vitamin D deficiency and calcium malabsorption.
†Signs and symptoms include bone pain, osteoporosis, kidney stones and neuropsychiatric manifestations. Additionally, patients with hyperparathyroidism due to chronic kidney disease may experience pruritus and calcification of soft tissues and vessels.
CKD, chronic kidney disease; HPT, hyperparathyroidism; PTH, parathyroid hormone.

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<th>Table 2</th>
<th>Clinicopathological correlates of primary hyperparathyroidism</th>
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<tr>
<td>Diagnosis</td>
<td>Parathyroid hyperplasia (multigland disease)</td>
</tr>
<tr>
<td><strong>Frequency</strong></td>
<td>~10–15%</td>
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<tr>
<td><strong>Onset</strong></td>
<td>Variable, but in familial forms earlier than single-gland disease</td>
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<tr>
<td><strong>Clinical features</strong></td>
<td>Variable symptom†, depending on the familial syndrome (25% of cases). Consider family history of hypercalcaemia (FHH) and/or other familial endocrine disorders (MEN)</td>
</tr>
<tr>
<td><strong>Biochemical features</strong></td>
<td>Variable biochemical profile (mild-to-severe hypercalcaemia and HPT) depending on the genetic syndrome. In hypocalciuria, consider FHH</td>
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<tr>
<td><strong>Radiological features</strong></td>
<td>Multigland disease may be detected with imaging but usually localising studies are more helpful in the setting of recurrent disease after most of the abnormal parathyroid tissue has already been removed.</td>
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<td><strong>Treatment</strong></td>
<td>Four gland parathyroidectomy explored followed by subtotal parathyroidectomy</td>
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<td><strong>Special considerations</strong></td>
<td>Genetic testing should be considered, unless identifiable cause such as chronic kidney disease</td>
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<tr>
<td><strong>Prognosis</strong></td>
<td>Risk of recurrent and/or persistent disease after surgery, especially in individuals with FHH-1</td>
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*Rare exceptions include case reports of parathyroid ‘double adenomas’ and carcinoma arising from a background of parathyroid hyperplasia.
†Symptoms of hyperparathyroidism include bone pain, renal colic (nephrolithiasis) and neurocognitive symptoms related to hypercalcaemia (weakness, easy fatigability, anxiety, depression, altered cognition).
FHH, familial hypocalciuric hypercalcaemia syndrome; HPT, hyperparathyroidism; HPT-JT, hyperparathyroidism-jaw tumour syndrome; MEN, multiple endocrine neoplasia syndromes; PTH, parathyroid hormone; US, ultrasound.
defined as high serum PTH and normal serum ionised calcium in the absence of known causes of secondary HPT, has been recently described. The epidemiology, natural history and management of hypercalcemic primary HPT remain unclear at this time; however, undoubtedly a subset of individuals will develop hypercalcaemia, while some show target organ alterations such as decreased bone mineral density. Secondary HPT presents with high serum PTH and normocalcemia or hypocalcaemia. Although it is initially and adaptively response to a variety of stimuli resulting mainly in extracellular hypercalcemia (vitamin D deficiency, chronic kidney disease, idiopathic hypercalcuria, calcium malabsorption), long-standing secondary HPT may eventually develop into autonomous HPT (ie, tertiary HPT). Similar to primary HPT, tertiary HPT is biochemically characterised by an elevation of both PTH and calcium in the serum. However, tertiary HPT is usually easily distinguished from primary HPT by a readily identifiable aetiology, which in most circumstances is advanced chronic kidney disease. In light of these findings, careful assessment of medication history (ie, lithium, thiazide), renal function, urinary calcium and family history is important to exclude secondary/tertiary and hereditary HPT, as their prognosis and management can differ significantly from that of sporadic primary HPT. Serum 25-hydroxyvitamin D levels should also be measured; not only is vitamin D deficiency a frequent cause of secondary HPT, it is commonly associated with primary HPT and can mask the patient’s hypercalcemia. Familial syndromes are reported in about 5% of patients with primary HPT. Clinical findings suggestive of hereditary HPT include early onset of disease (<30 years); family history of hypercalcemia; skin lesions (lipomas, facial angiofibromas, truncal collagenomas), pituitary adenomas and neuroendocrine tumours (including pheochromocytomas) associated with multiple endocrine neoplasia syndromes; jaw tumour with or without renal cysts (HPT-JT syndrome); hypercalcemia associated with hypocalciuria or a calcium-to-creatinine clearance ratio <0.01 (FHH). When familial disease is suspected, serum calcium measurement of first-degree relatives should be considered along with other clinical investigations. In cases where MEN-1 is suspected on the basis of multiglandular primary HPT with or without other concomitant MEN-1 associated tumours, testing for a MEN1 mutation is recommended. A definite diagnosis of FHH may ultimately require demonstration of a germline mutation in CASR, GNAS1 and 2P251. A severe neonatal phenotype (neonatal severe HPT) has also been described, presenting with severe HPT and life-threatening hypercalcaemia in the first six months of life. Although rare, parathyroid carcinoma should be considered in all patients with HPT and the following features: severe hypercalcaemia (albumin-corrected calcium >3 mmol/L), extremely high PTH levels (>3 times the upper limit of normal), concomitant bone and kidney involvement, palpable neck mass, jaw tumour, and family history of parathyroid or other endocrine disorders. Clinically, parathyroid carcinomas tend to present at an earlier age, with equal frequencies in both sexes. Biochemically, a third-generation/second-generation PTH ratio >1 has been shown to help predict parathyroid malignancy prior to surgery by correlating with other clinical, biochemical and radiological findings. Although this method has not been widely accepted, it has been proposed based on the fact that most parathyroid carcinomas overproduce amino-PTH, which is recognised by third-generation but not second-generation PTH assays. However, rare ‘non-functioning’ parathyroid carcinomas have also been reported. Radiographic lesions suspicious for distant metastasis may also be identified on sestamibi scintigraphy or CT scan, which can be confirmed on biopsy. RADIOLOGICAL INVESTIGATIONS In conjunction with clinical and biochemical findings, radiological investigations are necessary to guide treatment decision-making in primary HPT (tables 1 and 2). Extra-parathyroid imaging is performed to assess PTH-related complications on skeletal and renal structures, as positive findings may warrant surgery even in patients with asymptomatic primary HPT. For instance, renal ultrasonography is performed to exclude clinically ‘silent’ nephrocalcinosis and nephrolithiasis, which can occur in up to 20% of cases. Measurement of bone mineral density at the lumbar spine, hip and distal third of the forearm should also be investigated using dual-energy X-ray absorptiometry (figure 1A). The distal third of the forearm, enriched in cortical bone, is generally the first site to be affected because PTH preferentially affects cortical bone. The lumbar spine (rich in trabecular bone) is usually more preserved, whereas the hip region (mixture of cortical/trabecular bone) tends to show intermediate findings. However, it should be noted that a subset of patients (~15%) present with a different densitometry profile, with mainly vertebral osteopenia or osteoporosis. In patients with severe HPT, plain-film radiography and CT imaging may identify signs of bone disease such as pathological fractures, ‘salt and pepper’ appearance of the skull, tapering of the distal third of the clavicles, lytic lesions (figure 1B) of the pelvis, long bones and shoulders, and subperiosteal bone erosions in the distal phalanges. Parathyroid imaging is used to localise and characterise parathyroid abnormalities preoperatively. It is particularly helpful in the identification and characterisation of single-gland disease (adenoma or rare carcinoma) although it may also detect multigland parathyroid disease (usually hyperplasia) allowing for the appropriate surgical approach. In patients with severe HPT, plain-film radiography and CT imaging may identify signs of bone disease such as pathological fractures, ‘salt and pepper’ appearance of the skull, tapering of the distal third of the clavicles, lytic lesions (figure 1B) of the pelvis, long bones and shoulders, and subperiosteal bone erosions in the distal phalanges.

PARATHYROID IMAGING

In order to better understand the pathological manifestations of HPT (tables 1–3), the characteristics of normal parathyroid glands will also be highlighted here.
Normal parathyroid

In healthy individuals, the parathyroid glands are generally 4 in number (two superior, two inferior), although supernumerary parathyroid glands can occur in 2–13% of the population.\textsuperscript{38, 42-46, 120} It should be noted that all superior glands are located within the thyroid gland or in its pseudocapsule.\textsuperscript{121} Grossly, a normal parathyroid gland appears brown, round-to-ovoid, small (often $<$6–8 mm) and can weigh up to 40–60 mg each (figure 3).\textsuperscript{38–40, 42-44-46, 99, 120} Occasionally, parathyroid tissue can occur in ectopic locations (near the carotid bifurcation, pericardial sac, mediastinum, retroesophageal space, vagus nerve, angle of the jaw and thyroid gland) as a result of aberrant migration during embryogenesis.\textsuperscript{38-40, 42-44-46, 99, 120} Histologically, each gland is comprised of glandular parenchyma, adipose tissue and an arborising network of blood vessels, generally confined within a thin pseudocapsule.\textsuperscript{38, 42-44, 45}
The parenchymal cells are composed predominantly of chief cells (main producer of PTH), along with oncocytic cells (rich in mitochondria; also known as oxyphilic cells or oxyphils) and transitional oncocytic cells (representing an intermediate phase between chief cells and oncocytic cells).38–40 42 44–46

Handling of parathyroidectomy specimens and the role of intraoperative consultation

The clinical history, biochemical work-up, as well as number, size and weight of affected gland(s) are important parameters to consider when rendering a clinicopathological diagnosis of parathyroid proliferations. The initial examination should start with a gross inspection. The parathyroid gland(s) should be carefully weighed and measured after removal of the surrounding fat tissue.39 41 42 44 At this stage, it is critical to identify the vascular pole (hilum) before slicing the gland horizontally to this plane.39 41 42 This step is particularly important, given the fact that an atrophic rim of normal parathyroid tissue, one of the key histopathological features of a parathyroid adenoma,42 44 122 is often located at the vascular pole (hilum) of the gland.

Accurate diagnosis of parathyroid proliferations can be difficult or even impossible at the time of the intraoperative assessment.39 40 42 44 46 123 124 The primary role of the intraoperative pathological consultation is to confirm the presence of parathyroid tissue in the resected specimen and to exclude other tissues, such as lymph nodes or thymus, which can be mistaken for parathyroid tissue by the surgeon.35 39–46 123 125 In patients

### Table 3

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Parathyroid hyperplasia (multigland disease)</th>
<th>Parathyroid adenoma (single-gland disease)*</th>
<th>Parathyroid carcinoma (single-gland disease)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histopathological features</td>
<td>Enlarged cellular glands</td>
<td>Well-circumscribed tumour with atrophic rim of normal parathyroid tissue</td>
<td>Large tumour invading adjacent structures, unequivocal vascular invasion or perineural invasion, and/or metastasis</td>
</tr>
<tr>
<td>Ancillary biomarkers</td>
<td>Nodular hyperplasia can express same biomarkers as adenomas; but may also express galectin-3</td>
<td>Preserved expression of parafibromin, Rb, p27, Bcl-2a, mdm2, APC, and PGP9.5, no p53 overexpression, negative for galectin-3; Ki67 index usually &lt;5%</td>
<td>Loss of expression of parafibromin, Rb, p27, Bcl-2a, mdm2, APC, and overexpression of p53 (rare), PGP9.5, positivity for galectin-3, increased Ki67 index (&gt;5%)</td>
</tr>
<tr>
<td>Inheritance</td>
<td>Hereditary in &gt;25% of cases</td>
<td>Usually sporadic</td>
<td>Usually sporadic (80–90%), with the exception of HPT-JT as well as isolated familial hyperparathyroidism associated with germline HRPT2/CDC73 mutations</td>
</tr>
<tr>
<td>Molecular pathogenesis</td>
<td>Abnormal CaSR signalling, cyclin D1 and Wnt/β-catenin signalling</td>
<td>Abnormal CaSR signalling, cyclin D1 and Wnt/β-catenin signalling; MEN1, CCND1/PRAD1, CDKN1B, CDKis, CaSR, VDR, ZFX, CTNNB1, EZH2, POT1, APC, SFRPs, WT1, RIZ1, RASSF1A, HIC1, GSK3β, microRNA deregulation</td>
<td>Inactivation of parafibromin, abnormal cyclin D1 and Wnt/β-catenin signalling; CDC73/HRPT2, APC, PRUNE2, HIC1, GSK3β, mTOR, ML2, THRAP3, PIK3CA, CCND1/PRAD1, CDKN1B, CDKis, EZH2, RIZ1, RASSF1, MEN1, WT1, CaSR, microRNA deregulation, APOBEC mutational signature</td>
</tr>
<tr>
<td>Associated somatic alterations</td>
<td>Not defined</td>
<td>Not defined</td>
<td>Inherited inactivated allele of CDC73/HRPT2 in 10–20% of cases</td>
</tr>
</tbody>
</table>

*Rare exceptions include case reports of parathyroid ‘double adenomas’ and carcinoma arising in a background of parathyroid hyperplasia.

### References

with HPT, intraoperative confirmation of glandular abnormality (enlarged cellular gland) is also required (figure 4). In the past, pathologists were also asked to assess parenchymal-to-adipose cell ratio and to perform fat stains (oil red O or Sudan IV stains) to diagnose parathyroid proliferations. The value of these stains remains controversial, with most experts agreeing that this approach is not reliable in distinguishing parathyroid adenoma from hyperplasia. While the evaluation of a second parathyroid gland may be an alternative to fat stains, the recent development and adoption of rapid intraoperative PTH assay appears to be a more precise tool to distinguish adenoma from hyperplasia. In patients with single-gland disease, removal of a solitary gland results in reduction of PTH levels of >50%, often >75%, within 10 min. Consequently, many hospitals with high volumes of parathyroid surgeries use rapid intraoperative PTH measurements, immediately before and 10 min after resection. If a significant drop (>50%) in PTH levels is noted, no further exploration is undertaken. In this setting, a definite diagnosis of the parathyroid pathology is not required at the time of surgery. Immediate hypocalcaemia after surgery is frequently seen in patients with single-gland parathyroid disease and should be monitored accordingly.

Histopathological correlates of HPT
Primary HPT can occur hereditarily (~5%) or sporadically (~95%) due to parathyroid hyperplasia, adenoma or carcinoma (table 3). With the exception of rare double adenomas, parathyroid adenomas and carcinomas are almost always uniglandular (solitary) lesions, whereas hyperplasia represents multiglandular proliferation, which can be asymmetrical and asynchronous. The presence of an atrophic rim of hypocellular non-tumorous parathyroid tissue adjacent to a cellular proliferation often allows an accurate diagnosis of ‘parathyroid adenoma’ in the appropriate clinical and biochemical setting (figure 5). However, in the absence of an atrophic rim, hyperplasia cannot be excluded at the morphologic level. For this reason, an enlarged parathyroid gland, lacking a normal rim, should be classified as an ‘enlarged cellular parathyroid gland’. If biochemical cure is achieved after surgery, a diagnosis of parathyroid adenoma can be rendered, after excluding parathyroid carcinoma. It should be noted that hyperplastic glands can also present small areas of normocellular or slightly cellular tissue indistinguishable from those surrounding parathyroid

Figure 3  Normal parathyroid gland. A normal parathyroid gland appears grossly brown and round-to-ovoid. It measures less than 6–8 mm and weighs up to 40–60 mg each.

Figure 4  The role of intraoperative consultation in hyperparathyroidism. The primary role of the intraoperative pathological consultation is to confirm the presence of parathyroid tissue and to exclude other tissues mimicking parathyroid gland. The parenchymal-to-adipose cell ratio is not a reliable tool to distinguish an abnormal gland. In patients with hyperparathyroidism, intraoperative confirmation of an abnormal gland based on the weight (>60 mg) and size (>8 mm) of the gland is also required. Accurate diagnosis of parathyroid proliferations can be difficult or even impossible at the time of the intraoperative consultation. Thus, the term ‘enlarged cellular gland’ should be applied to abnormal gland at the time of intraoperative consultation. The rapid intraoperative parathyroid hormone assay appears to be a more precise tool to distinguish adenoma from hyperplasia at the time of intraoperative consultation.

Figure 5  Parathyroid adenoma. With the exception of rare double adenomas, parathyroid adenomas are almost always uniglandular (solitary) lesions. The presence of an atrophic rim of hypocellular non-tumorous parathyroid tissue adjacent to a cellular proliferation often allows an accurate diagnosis of ‘parathyroid adenoma’ in the appropriate clinical and biochemical setting.
adenomas.\textsuperscript{38-40,42-44,46,99,123,131} Therefore, correlation with intraoperative PTH levels and postoperative PTH and calcium levels is crucial to render a definitive diagnosis of adenoma.\textsuperscript{38-46,99,123,131}

Parathyroid adenomas are by far the most common cause of primary HPT, accounting for 80–85% of cases.\textsuperscript{4,38-42,46} Foci of cystic change can occur in larger adenomas, particularly in the setting of a familial syndrome (ie, HPT-JT syndrome).\textsuperscript{43,44,123} Cystic change can occur in larger adenomas, particularly in long-standing prominent hyperplasia related to chronic renal failure.\textsuperscript{38-40,42-44,45,99,133} While ‘chief cell’ adenomas are the most prevalent type, rare morphological variants have also been reported, including oncocytic cell adenomas, water-clear cell adenomas and lipoadenomas.\textsuperscript{38,40,42-44,45,99,133} Rare cases with spindle cell features have also been described.\textsuperscript{42} Various architectural patterns have been reported, including arrangements in cords, nests, sheets, papillary, pseudopapillary and follicles with palisaded appearance around blood vessels (figure 6).\textsuperscript{38-40,42-44,45,99,133} Some parathyroid adenomas show prominent follicular architecture, mimicking thyroid tissue (figure 6).\textsuperscript{38-40,42-44,45,99,133} However, a distinction can be made based on the observation that the follicles in parathyroid adenomas typically do not contain birefringent calcium oxalate crystals, which are usually seen in thyroid follicles.\textsuperscript{42,99,134} Cytologically, the nuclei of tumour cells often appear round and larger than those of adjacent non-neoplastic cells.\textsuperscript{38-40,42,44-46,99,133} Occasionally, the use of immunohistochemistry (positivity for PTH, GATA3 or GCM-2) is helpful to confirm the primary parathyroid origin of an unusual neoplasm (figure 6).\textsuperscript{44} Although scattered mitotic figures can be present, the proliferative index of most adenomas, as assessed by Ki-67 (MIB-1), is generally <5%.\textsuperscript{38-40,42-44,46,98,99,133,135} The individual hyperplastic glands are grossly increased in size and weight; microscopically, they show chief, oncocytic and/or clear cells.\textsuperscript{38-40,42-44,45,99} In most cases, chief cells tend to predominate, hence the classic term ‘chief cell hyperplasia’. In rare instances, abundant stromal fat is observed, resulting in a morphological variant termed ‘lipohyperplasia’.\textsuperscript{42,40-42,44,45,99} Foci of cystic change with fibrosis and hemosiderin deposition can occur, particularly in long-standing prominent hyperplasia related to chronic renal failure.\textsuperscript{38-40,42-44,45,99} A rare ‘water-clear cell’ variant of parathyroid hyperplasia has also been reported, presenting with more florid HPT and hypercalcaemia than the common chief cell hyperplasia, often with grossly, enlarged, cystic and haemorrhagic glands.\textsuperscript{38-40,42-44,45,99} Similarly, water-cell adenomas have also been described.\textsuperscript{38-40,42,44,45,99,136-139} The glycogen-free water-clear cell morphology is attributed to

Figure 6 Various cytological and architectural patterns associated with parathyroid adenomas. Most adenomas consist of chief cells (A), rare morphological variants have also been reported, including oncocytic cell adenomas, mixed cellular adenomas (B), water-clear cell adenomas (C), and lipoadenoma. Various architectural patterns have been reported, including arrangements in cords, nests, sheets, papillary/pseudopapillary (D) and follicles (E). Some parathyroid adenomas show prominent follicular architecture and proteinaceous material in the lumina, mimicking thyroid tissue (E). However, parathyroid proliferations with follicular growth typically lack birefringent calcium oxalate crystals, which are often seen in thyroid follicles. Occasionally, the use of immunohistochemistry (positivity for parathyroid hormone (PTH), GATA3 or GCM-2) is helpful to confirm the primary parathyroid origin in challenging cases (F; PTH immunostain).
cytoplasmic clearing due to the presence of numerous small vacuoles derived from the Golgi apparatus.

The molecular basis underlying this ‘water-clear cell’ variant morphology remains unclear at this time. Rare cases of ‘double adenomas’ have also been described to cause multiglandular disease in primary HPT. Since the morphological distinction between adenomas and nodular parathyroid hyperplasia is somewhat arbitrary at times, there is controversy as to whether double adenomas are truly a distinct clinical and biological entity from nodular hyperplasia. Furthermore, emerging molecular data suggest that the nodules in parathyroid nodular hyperplasia represent clonal proliferations that are more closely related to adenomas, making the distinction between multiple adenomas and nodular hyperplasia even more ambiguous. In fact, this is best demonstrated in patients with MEN syndromes (MEN-1, MEN-4), as well as those with tertiary HPT, who present with multiglandular adenomas; these tumours represent distinct clonal proliferations on molecular studies but are clinically thought to be hyperplasia due to multiglandular involvement. In light of these findings, the term ‘nodular parathyroid disease’, rather than nodular hyperplasia, may seem more appropriate to reflect the molecular basis of these clonal proliferations arising in a background of polyclonal diffuse hyperplasia. In other terms, this terminology may seem more accurate when designating neoplastic proliferations involving multiple parathyroid glands.

Secondary HPT is generally caused by diffuse parathyroid hyperplasia in response to prolonged reduced levels of extracellular calcium from various secondary aetiologies. In late stages, the emergence of an autonomous PTH-producing neoplasm (usually adenomas or rare carcinoma) from a background of secondary hyperplasia has been described, causing ‘tertiary hyperparathyroidism’. Clinically, this histopathological progression from diffuse to nodular parathyroid hyperplasia is associated with the development of refractory HPT and new hypercalcaemia, in patients with previously controlled secondary HPT. At the molecular level, this progression sequence has been linked to clonal transformation from diffuse polyclonal hyperplasia due to decreased calcium-sensing receptor (CaSR) signalling from hypocalcaemia. Morphologically, parathyroid hyperplasia with prominent nodular growth pattern may show areas of haemorrhage, cyst formation, fibrosis and chronic inflammation, which often correlate with the degree of HPT. Cytologically, chief cells tend to predominate in early phases of secondary HPT, with gradual replacement by oncocyctic cells described in late stages of the disease.

While most parathyroid tumours are benign neoplasms (adenomas), parathyroid carcinomas can occur in <1–5% of patients with HPT. Their cytological composition is similar to parathyroid adenomas, with varying proportions of chief cells, oncocyes, transitional oncocyes and scattered water-clear cells. Most parathyroid carcinomas show mild-to-moderate nuclear atypia, which is indistinguishable from...
adenomas. Overall, the distinction between parathyroid carcinomas and adenomas is extremely difficult on cytology, with the exception of a subset of carcinomas that exhibit significant nuclear pleomorphism with macronucleoli. A triad of high-risk histopathological features (necrosis, macronucleoli, >5 mitoses per 50 high-power fields) has been reported to predict malignant behaviour in some parathyroid tumours. ‘Atypical adenomas’ remain a controversial pathological entity in medicine. In the parathyroid glands, these represent borderline neoplasms showing some histopathological findings of parathyroid carcinomas (band forming fibrosis, increased mitotic activity, presence of tumour cell within a thickened capsule) but lacking the definite diagnostic features of malignancy (invasion into adjacent tissues, vascular invasion and/or metastases). The practising pathologist should be aware of the fact that fibrosis can occur in many situations including previous manipulations (post-FNA biopsy for PTH measurements or cytological examination, or ethanol injection), MEN1/MEN4 syndrome, lithium intake or long-standing chronic renal failure related-parathyroid hyperplasia, in the setting of parathyroiditis or in large degenerated adenomas. While the parathyroids do not have a true anatomic capsule, fibrotic bands at the periphery of the gland can mimic tumour cells within a thickened capsule. As discussed earlier, mitotic figures can be present in some benign parathyroid disease. In reality, the concept of ‘atypical parathyroid adenoma’ probably stems from the previous limits of the histopathological examination, whereby borderline parathyroid tumours with ‘equivocal’ histological features could not be classified with certainty based on morphology alone, given significant inter-observer variability as well as the lack of clinicopathological correlations in many practices. However, with recent developments in molecular pathology and ancillary tools, the classification of these borderline tumours has been greatly refined. When used in the appropriate clinical and pathological setting, ancillary biomarkers serve a crucial role in the distinction of malignancy in these borderline tumours, thereby enhancing the accuracy of the pathological examination. In diagnostically challenging cases, the use of ancillary biomarkers is extremely valuable to support or exclude a diagnosis of malignancy, while uncovering differences in molecular phenotypes and prognosis. Based on the currently available evidence and our own experiences, loss-of-expression of retinoblastoma protein (Rb), Bcl-2a, p27, parafibromin, mdm2 and APC, as well as increased MIB-1 (Ki67) proliferative index >5%, overexpression of p53 and positivity for galectin-3 (in the absence of multiglandular disease) favours a diagnosis of
malignancy in a parathyroid neoplasm with worrisome histopathological features. In particular, the use of parafibromin immunostain (figure 8E) is very helpful to differentiate between parathyroid adenomas (intact nuclear and nucleolar parafibromin expression) and parathyroid carcinomas (complete loss of nuclear or nucleolar parafibromin expression). 38 42 43 46 98 99 131 153 156 158–164 Recently, routine assessment of parafibromin staining has been proposed in all parathyroid carcinomas to help select patients for genetic testing and to predict prognosis: those with parafibromin-negative parathyroid carcinomas had a significantly higher risk of recurrence, a decreased 5-year survival of 59% and a decreased 10-year survival of 23%, which may warrant closer surveillance.

Parathyromatosis is a rare cause of persistent or recurrent HPT after parathyroidectomy. 39 42 44 157 165 Some authors believe that this entity represents implantation of benign parathyroid tissue that spilled during surgery, whereas others believe that it reflects local recurrence and/or parathyroid malignancy. 39 42 44 157 165 Clinical information, presence or absence of invasive growth (especially angioinvasion) and ancillary tools may be helpful in making this distinction. 39 42 44 157 165

PATHOGENESIS AND MOLECULAR GENETICS OF HPT
Normal physiology of PTH secretion
In normal physiology, PTH secretion is mediated by the CaSR signalling pathway. 7 33 80 88 141 166–169 CaSR, encoded by CaSR gene, is a G-protein-coupled receptor found on the surface of parathyroid chief cells. In the presence of circulating calcium, CaSR is activated and recruits phospholipase C β, through Gq and G11. 7 33 80 88 141 166–169 This leads to a complex series of reactions involving production of inositol triphosphate, release of calcium from intracellular stores, elevation of diacylglycerol concentrations, activation of protein kinase C, phosphorylation and internalisation of CaSR. 7 33 80 141 166 169 Ultimately, activation of CaSR signalling pathway suppresses PTH secretion by two mechanisms: first, CaSR signalling activates Gqg and phospholipase A2, generating arachidonic acid metabolites, which have been shown to inhibit PTH secretion directly; second, vitamin D receptor (VDR) is overexpressed on parathyroid chief cells, thereby increasing their sensitivity to the negative feedback exerted by 1,25(OH)2-vitamin D, further suppressing PTH production. 7 33 80 141 168 169 When hypocalcaemia occurs, CaSR signalling is downregulated, and this allows the parathyroid chief cells to release the PTH stored in their cytoplasmic granules, while concurrently increasing PTH gene transcription and protein synthesis. 7 33 80 141 168 169 PTH binds to PTH/PTH-related peptide receptors at various sites in the body, stimulating osteoclast-mediated bone resorption, renal reabsorption of calcium and 1,25(OH)2D synthesis to increase calcium levels. 7 33 80 141 168 169 With the rise of circulating calcium levels, a classic endocrine feedback loop occurs, whereby the CaSRSs and its signalling pathway are reactivated, regulating PTH secretion. 7 33 80 141 168 169 In HPT, various alterations inactivating CaSR signalling pathway can occur, causing excess PTH secretion. 7 33 80 141 168 169 Furthermore, if downregulation of CaSR persists over time, studies have shown that it can lead to aberrant cell growth and proliferation (thought to be related to aberrant Wnt/β-catenin and cyclin D1 signalling), suggesting that CaSR signalling may also serve a tumour suppressor function in the parathyroid glands. 7 11 33 42 78 81 141 152 166 170–172

Hereditary HPT
Most patients with inherited parathyroid disease present with multiglandular involvement (ie, parathyroid hyperplasia), although rare solitary proliferations can also occur. 30 38 42 46 99 143 Common hereditary syndromes associated with HPT include familial cancer predisposition syndromes (MEN-1, MEN-2A, MEN-4 and HPT-JT syndromes) and hereditary ‘hypercalcemic’ syndromes related to aberrant CaSR signalling (FHH-1, FHH-2, FHH-3, neonatal severe HPT and familial hypercalcemic hyperparathyroidism). 13 32 39 42 43 46 82 99 141 161 162 Rarely, inherited HPT can occur in a non-syndromic fashion, also known as ‘familial isolated hyperparathyroidism’, due to germline mutations of MEN1, CDC73/HRPT2, CaSR or cyclin-dependent kinase inhibitor (CDKI)-encoding genes (CDKN1B, CDKN1A, CDKN2B and CDKN2C). 33 39 42 99 141 173

MEN syndromes represent a group of autosomal-dominant cancer predisposition syndromes. 7 32 42 141 143 174 Three distinct types (MEN-1, MEN-2A, MEN-4) are associated with HPT. 32 42 141 143 MEN-1 syndrome is caused by germline inactivating mutations in the tumour suppressor gene MEN1 (11q13), encoding ‘menin’ protein, which is thought to prevent nuclear translocation of β-catenin, thereby suppressing Wnt/β-catenin signalling, a well-known tumorigenesis pathway. 32 42 141 143 174 Phenotypically, MEN-1 is characterised by the development of multiglandular parathyroid adenomas (90%), gastroenteropancreatic neuroendocrine tumours (60%) and pituitary adenomas (30%); additional tumours reported in this syndrome include adrenocortical tumours, facial angiofibromas, collagenomas, lipomas and/or other neuroendocrine tumours of various sites (thymus, lung, stomach). 32 42 85 99 141 143 174 The mechanisms underlying tumorigenesis in this syndrome appear to be related to somatic inactivation of the wild-type allele, in addition to an inherited altered copy of the MEN1 gene. 32 42 85 99 141 143 176

Recently, a subset of patients presenting with multiglandular parathyroid disease and what appeared to be MEN-1 phenotype but lacking MEN1 gene mutation were subsequently given the diagnosis of MEN-4 syndrome (also known as ‘MEN X syndrome’). 42 85 98 99 141 143 176 These patients harbour inactivating germline mutations in the tumour suppressor gene CDKN1B (12p13.1; encoding the CDKI, p27kip1, implicated in cyclin D1 signalling). 42 85 98 99 141 143 176 Phenotypically, patients with MEN-4 are almost indistinguishable from those with MEN-1 related disease. 4 32 42 85 98 99 141 143 176 On pathological examination, both MEN-1 and MEN-4-related parathyroid proliferations may show regions of fibrosis and nodularity. 39 42 44 46 85 99 176

MEN-2A syndrome is characterised by activating germline mutations in the RET proto-oncogene (10q11.2), predisposing to the development of HPT (20–30%), medullary thyroid carcinoma arising from a background of precursor C-cell hyperplasia (also known primary or neoplastic C-cell hyperplasia) and pheochromocytomas arising in adenral medullary hyperplasia. 4 32 42 44 46 99 141 143 177 178

HPT-JT is another autosomal-dominant cancer predisposition syndrome associated with HPT due to germline inactivating mutations of CDC73 (formerly known as HRPT2; 1q31.2). 4 32 42 51 141 158 161 162 179 Phenotypically,
HPT-JT syndrome is characterised by primary HPT, as well as fibro-osseous lesions in the mandible and maxilla, and occasional urogenital and renal lesions, including cysts, hamartomas, renal cell carcinoma and Wilms’ tumours.4 7 32 141 158 161 The HPT is usually attributed to multiglandular parathyroid disease, comprising of multiple parathyroid nodules/adenomas associated with cystic change; about 10–20% of these lesions eventually progress to parathyroid carcinomas, often due to bi-allelic inactivation of CDC73/HIPPI2 and complete loss of parathymin expression.4 31 42 51 141 158 In addition to the previously described cancer predisposition syndromes, a group of familial ‘hypercalcemic’ syndromes (FHH-1, FHH-2, FHH-3, neonatal severe HPT, familial hypercalcic hypercalcaemia) has been reported to cause HPT, due to aberrant inactivation of CaSR signalling in the parathyroid glands, kidneys and skeletal bones.13 32 42 75 76 77 141 These conditions are generally inherited in an autosomal-dominant manner and appear to have near-complete penetrance for the phenotypical expression of hypercalcaemia.13 32 42 75–77 141 167 Among these, FHH type 1 is the most classic form, characterised by germline heterozygous inactivating mutations of CaSR (3q21.1), encoding CaSRs.13 32 42 75–77 141 167 Clinically, affected patients are typically asymptomatic and present with inappropriate hypercalcinaemia in the setting of overt hypercalcaemia and normal or elevated serum PTH.7 13 30 32 42 75–77 141 167 At the molecular level, this phenomenon is mainly attributed to mutant CaSR in the kidney, preventing the normal physiological hypercalcic response to hypercalcaemia.7 13 30 32 42 75–77 141 167 Although concurrent primary HPT due to a parathyroid adenoma can occur, the majority of patients with FHH-1 do not have detectable parathyroid disease, and therefore rarely benefit from parathyroidectomy.7 13 29 30 34 42 141 180 181 Pathologically, parathyroid enlargement (hyperplasia) has been reported in 15–20% of cases.3 32 42 44 83 183 In addition to FHH-1, a severe neonatal phenotype (neonatal severe primary HPT) has been described to cause multiglandular parathyroid disease due to germline homozygous inactivating mutations of the CaSR gene.4 7 13 29 42 141 Clinically, patients with this condition typically present shortly after birth, with life-threatening hypercalcemia, hypercalcicuria and severe HPT.4 7 13 29 30 42 141 In contrast to FHH-1, neonatal severe HPT generally requires urgent total parathyroidectomy to prevent a fatal outcome in affected patients.5 34 34 36 On pathological evaluation, the parathyroid glands are visibly enlarged, hyperplastic and hypercellular.4 7 13 30 32 42 44 46 A milder form, known as familial hypercalcic hypercalcaemia (or autosomal-dominant moderate HPT), has also been reported to cause HPT due to germline inactivating mutation in the intracytoplasmic tail domain of the CaSR gene.13 30 42 141 184 Clinically, affected patients present with elevated calcium and PTH levels, an appropriate hypercalcic response, hypermagnesaemia and hyperphosphaturia.13 30 42 141 184 Normalisation of calcium levels has been described in some cases after subtotal parathyroidectomy.13 30 42 44 141 184 Pathologically, multiglandular parathyroid disease (ie, nodular hyperplasia) is observed in most cases.

Recently, two novel forms of FHH (types 2 and 3) have been uncovered.13 32 38 88 90 91 141 185 FHH-2 and FHH-3 are associated, respectively, with germline inactivating mutations of GNA11 (19p13.3) and AP2SI (19q13.2) genes.13 32 88 90 91 141 185 GNA11 gene encodes the α-subunit of G11, one of the principal G proteins activating CaSR signalling pathway, whereas AP2SI gene encodes the adaptor protein 2 σ-subunit involved in CaSR endocytosis.13 32 88 90 91 141 185 Both mutations in GNA11 and AP2SI genes cause hypocalciuric hypercalcaemia though aberrant inactivation of CaSR signalling, similar to FHH-1.13 32 88 90 91 141 185 Phenotypically and pathologically, patients with FHH-2 typically present with features indistinguishable from FHH-1, whereas those with FHH-3 tend to present with osteomalacia, higher levels of PTH and more frequent parathyroid gland involvement (hyperplasia).3 11 32 42 88 90 91 141 183

Sporadic parathyroid adenomas
Most parathyroid adenomas are sporadic neoplasms.39 42–44 141 Although the majority of cases do not have an identifiable risk factor, epidemiological studies suggest that a previous history of ionising radiation predisposes to the formation of sporadic parathyroid tumours.4 7 38 42 Somatic alterations in MEN1 and CCND1/PRADI genes have emerged as important drivers in the development of 25–40% of sporadic parathyroid adenomas.42 46 81 141 186 187 As discussed previously, MEN1 and its gene product, menin, are thought to serve a tumour suppressor role by regulating Wnt/β-catenin signalling, a well-documented pathway in tumorigenesis.141 175 186 Although an inherited mutant copy of the MEN1 gene may be sufficient to cause multiglandular parathyroid disease (hyperplasia) and HPT (in MEN-1 syndrome or isolated familial HPT), an additional somatic inactivating mutation in the remaining copy of MEN1 (ie, the wild-type allele) is generally required for the development of sporadic parathyroid tumours (>25% in some series).32 81 141 Alternatively, pure somatic mutations can result in bi-allelic inactivation of MEN1, seen in some parathyroid adenomas.141 189 190 These findings are consistent with comparative genomic hybridisation reports, where loss of 11q ( locus of MEN1 ) is the most frequent alteration in adenomas; losses of 1p, 6q, 9p, 11p, 13q and 15q (possible loci for undiscovered tumour suppressor genes) and gains in 7, 16p and 19p ( possible loci for undiscovered oncogenes ) have also been reported.42 141 191 CCND1/PRADI (11q13) is a proto-oncogene, encoding cyclin D1 protein, a holoenzyme thought to inactivate the tumour-suppressor retinoblastoma protein (Rb), inducing cellular proliferation.31 33 80 81 141 192–196 Somatic activating alterations of CCND1/PRADI gene, causing excess cyclin D1 signalling, have been reported in 20–40% of sporadic parathyroid adenomas.31 33 80 81 141 192–196 Deregulation of CCND1/PRADI gene expression can occur as a result of rearrangements with PTH promoter gene (∼8%) and/or other enhancers/promoters, leading to amplification or transcriptional activation of cyclin D1.31 33 38 46 80 141 192–196

Concurrent to the discovery of germline mutations in CDKI-encoding genes causing familial HPT (in MEN-4 syndrome and isolated familial HPT), somatic alterations of CDKI-encoding genes (CDKN1B, CDKN1A, CDKN2B, CDKN2C) were also identified in sporadic parathyroid adenomas.31 33 38 46 80 81 141 173 197 Of these, somatic inactivating mutations of the CDKN1B gene (encoding p27Kip1) causing excess cyclin D1 signalling have been well described, and this finding was further corroborated by studies showing decreased expression of p27 in sporadic parathyroid adenomas at both the RNA and protein levels, supporting its role in parathyroid tumorigenesis.42 141 197–199

Despite its well-documented role in hereditary HPT, somatic mutations of the CaSR gene have not been identified in sporadic disease.13 42 76 141 152 200 Nonetheless, several studies reported that a significant subset of sporadic parathyroid adenomas display aberrant CaSR signalling, characterised by reduced expression of VDRs and CaSRs.13 42 76 141 150 152 172 200–204

The exact mechanism underlying loss of expression of VDR and CaSR remains unclear, although genetic and epigenetic alterations in their respective genes have been sought but not found.\textsuperscript{13} 42 76 141 150–152 172 200–204 Given the fact that the pathogenesis of >50% of sporadic parathyroid adenomas remains unknown, these findings certainly raise the possibility that undiscovered somatic alterations, causing deregulation of CaSR signalling (either upstream or downstream of CaSR and VDR), may play an important role in parathyroid tumorigenesis.\textsuperscript{13}

The recent use of whole-exome sequencing in parathyroid adenomas revealed additional somatic mutations, involving ZFX (~5%); a putative proto-oncogene thought to be a downstream target of cyclin D1); CTNNB1 (2–5%; encoding β-catenin); EZH2 (~1%); a putative oncogene involved in histone methyltransferase activity and thought to cause aberrant accumulation of β-catenin) and POTT1 (<1%; regulator of telomere integrity and genome stability).\textsuperscript{82}

Over the past few years, DNA methylation and microRNA profiling studies have uncovered important epigenetic alterations in sporadic parathyroid tumours.\textsuperscript{141} 152 205 210–212 When compared to normal parathyroid tissue, parathyroid neoplasms were shown to have aberrant hypermethylation of tumour suppressor genes in Wnt/β-catenin signalling (APC, SFRP1, SFRP2, SFRP4), cyclin D1 signalling (CDKN2A, CDKN2B), as well as WTI, RIZ1, RASSF1A and HIC1.\textsuperscript{141} 152 205 210–211 As mentioned previously, cyclin D1 signalling plays an important role in cell cycle progression in parathyroid tissue and can be induced by Wnt/β-catenin signalling, another tumorigenesis pathway in many solid tumours.\textsuperscript{81} 141 196 212 214–216 APC, SFRP1, SFRP2 and SFRP4 genes all serve important regulatory functions in Wnt/β-catenin signalling, whereas CDKN2A and CDKN2B genes appear to regulate cyclin D1 signalling.\textsuperscript{81} 141 212 214–216 WTI (Wilms tumour 1) encodes an important transcription factor for cellular development and survival, and mutations in WTI have been linked to various solid tumours.\textsuperscript{81} 141 210 212 Similarly, RIZ1 (retinoblastoma-interacting zinc finger gene) appears to be involved in cell cycle regulation.\textsuperscript{141} 205 210 212 213 RASSF1A (Ras association domain family 1 isoform A; 3p21) is a component in the Ras signalling pathway and has been shown to suppress cyclin D1,\textsuperscript{218} 219 220 HIC1 (17p13) is a putative tumour suppressor gene, epigenetically deregulated in parathyroid tumours due to histone H3 lysine modification.\textsuperscript{211} 212 Recently, aberrant expression of a panel of embryonic-related microRNAs has been described in a series of sporadic parathyroid tumours, suggesting a role for microRNA deregulation in parathyroid tumorigenesis.\textsuperscript{211} 212

Sporadic parathyroid carcinomas

In contrast to parathyroid adenomas, parathyroid carcinomas are aggressive sporadic neoplasms, with significant morbidity and mortality related to PTH-mediated hypercalcaemia.\textsuperscript{7} Consequently, the study and understanding of their molecular biology is important to uncover novel diagnostic, prognostic and predictive biomarkers to improve patient care. With recent advances in molecular pathology, a wide array of genetic and epigenetic alterations, as well as biomarkers, have been uncovered in parathyroid cancer.\textsuperscript{31} 42 98 141 150 156 158 163 210 216

Inactivation of CDC73/HRPT2 (1q31.2) and its gene product, parafibromin, is a major driver in parathyroid cancer, appearing in >70% of cases in some series.\textsuperscript{31} 98 141 156 158 161 As mentioned previously, parafibromin serves critical tumour suppressor functions in normal parathyroid tissue, notably by interaction with PAF1 complex and regulating cyclin D1 and Wnt/β-catenin signalling.\textsuperscript{31} 42 98 141 156 158 161 221 It should be noted that decreased expression of parafibromin can occur in rare parathyroid adenomas (often with cystic features; associated with germ-line CDC73/HRPT2 mutations).\textsuperscript{42} 46 98 141 156 158 161 221 However, complete loss of parafibromin expression, due to bi-allelic inactivating mutations of CDC73/HRPT2, is almost pathognomonic for parathyroid carcinomas as it is almost never seen in adenomas.\textsuperscript{42} 46 98 141 156 158 161 221–223 Furthermore, all patients with histologically proven parathyroid carcinomas with loss of parafibromin staining should be considered for genetic testing since up to 20% of cases may harbour germline mutations in CDC73/HRPT2 with or without clinical features of HPT-JT syndrome.\textsuperscript{13} 31 42 43 81 141 151 164 205 210 211 212 219

Additional molecular alterations identified in parathyroid carcinomas (CCND1, CDKIs, MEN1, EZH2, RIZ1, RASSF1, GSK3B, WTI, APC, SFRP4, HIC1, CaSR, VDR and microRNA deregulation) are non-specific and can also occur in parathyroid adenomas.\textsuperscript{13} 31 42 43 81 141 151 164 205 210 211 212 219 Of note, MEN1 mutations, one of the key drivers in parathyroid adenomas (20–40%), appear to be less frequent in carcinomas (10–15%).\textsuperscript{31} 42 81 141 225–227 Recently, whole-genome sequencing uncovered additional mutations in parathyroid carcinomas, implicating PRUN2 (~18%), mTOR, ML2, THRAP3 and PIK3CA genes.\textsuperscript{141} 226 227 Overexpression of PGP9.5 (gene product of UCHL1 gene), galectin-3 and TERT (telomerase reverse transcriptase gene) were also described in some parathyroid carcinomas.\textsuperscript{160} 223 230 Previous comparative genomic hybridisation and molecular allelotyping studies on parathyroid carcinomas also revealed losses on chromosomes 1p, 3, 13q and 14 and gains on chromosomes 1q and 16.\textsuperscript{42} 141 Interestingly, some of these loci (1p, 13q, 16) were also reported in adenomas.\textsuperscript{141} 191 Several studies suggest a possible involvement of the p53/Rb signalling pathway: while TP53 mutations are rare, allelic loss and abnormal expression of p53 and retinoblastoma protein have been uncovered in a subset of parathyroid carcinomas.\textsuperscript{81} 98 141

TREATMENT AND PROGNOSIS

The management of HPT depends largely on its aetiology.\textsuperscript{1} 4 6 7 29 34 35 36 50 70 107 231 After excluding reversible secondary aetiologies, first-line treatment of irreversible secondary HPT (often related to chronic kidney disease) mainly involves medical therapies, using low-phosphate diet, phosphate binders, 1,25-dihydroxyvitamin D3 (calcitriol or analogues; to suppress PTH secretion) and/or calcimimetic agent (cinacalcet; an allosteric activator of CaSR).\textsuperscript{1} 4 6 7 11 29 34–36 50 72 74 107 231–234

Although percutaneous ethanol ablation may be an alternative to parathyroidectomy in select cases of primary HPT,\textsuperscript{232} 233 surgical excision of abnormal ‘hyperfunctioning’ parathyroid gland(s) remains the only curative treatment.\textsuperscript{1} 4 6 7 29 34–36 231 While symptomatic cases are addressed with parathyroidectomy, surgery in asymptomatic HPT is typically reserved to patients with one of the following conditions: age (<50 years), kidney disease (creatinine clearance <60 mL/min; radiographic nephrolithiasis/nephrocalcinosis; 24-h urinary calcium >400 mg/d with increased stone risk), bone disease (osteoporosis, ie, bone mineral density T score <−2.5 at distal 1/3 of radius, femoral neck, total hip, lumbar spine; radiographic vertebral fractures), overt hypercalcaemia (serum calcium >1.0 mg/dL above upper
In all cases of primary HPT, an underlying genetic syndrome should be considered, especially if hypocalcemia or multiglandular parathyroid disease is detected. In particular, familial hypocalcemic hypercalcaemia should be excluded, using urinary calcium-to-creatinine clearance ratio and/or genetic testing (CASR, GNA11, AP2SI), given the fact that most patients with this condition do not benefit from parathyroidectomy. Furthermore, patients with MEN-1, MEN-2A or familial isolated HPT merit closer monitoring, given their increased risk of recurrent/persistent disease due to multigland parathyroid involvement. Sporadic primary HPT is usually caused by single-gland parathyroid disease, commonly from a solitary adenoma. Minimally invasive parathyroidectomy is an increasingly popular approach, although open surgery with bilateral cervical exploration is still advocated by some endocrine surgeons, particularly in multigland disease and/or recurrent HPT. The complete removal of abnormal parathyroid tissue should be confirmed biochemically with intraoperative (or postoperative) PTH measurements. Following surgery, most patients with clinically and pathologically confirmed parathyroid adenoma are cured.

In parathyroid carcinomas, an oncological surgical approach, including at least en bloc resection (either as a primary or revision surgery), is paramount for disease control. Adjuvant radiation has been proposed to decrease local recurrence rate, although this approach remains controversial. Given its rarity, challenging diagnosis and high-risk of recurrence, suspected cases of parathyroid carcinomas should be referred to endocrinologists, surgeons and pathologists with extensive experience in endocrine oncology. At our institution, in a cohort of 16 patients with parathyroid cancer, the 5-year and 10-year disease-specific survival rates were 100% and 80%, respectively; the 5-year and 10-year disease-free survival rates were 69% and 43%. These findings confirm the relative indolent behaviour of parathyroid malignancies reported in the literature, while reinforcing the high risk of disease recurrence, which can occur many years after initial treatment.

In patients diagnosed with a parathyroid carcinoma, the tumour should be further tested for loss of parafibromin expression. Those showing loss of immunohistochemical parafibromin expression should be offered genetic testing for germline CDC73/HRPT2 mutations. In patients with primary HPT who cannot or do not want to undergo surgery, ethanol injection and alternative treatment modalities remain limited at this time. Emerging medical therapies, similar to those used in secondary HPT, have been shown to be beneficial, although not curative, in a subset of patients.

**CONCLUSION**

HPT is a common, morbid yet fascinating disorder in the evolving field of endocrinology. Clinico-pathological correlates of primary HPT include parathyroid adenoma (80–85%), hyperplasia (10–15%) and carcinoma (<1–5%). The clinical and biochemical history, including intraoperative PTH measurements, number, size and weight of the affected gland(s), are crucial parameters to consider when rendering a clinico-pathological diagnosis of parathyroid proliferations. Ultimately, the role of the pathologist pertains to the accurate distinction between single-gland (usually a solitary parathyroid adenoma) and multigland disease (diffuse and/or nodular parathyroid hyperplasia), all the while excluding a primary parathyroid malignancy. New developments in diagnostic imaging and intraoperative rapid PTH assays have substantially improved our ability to distinguish between single-gland and multigland parathyroid disease preoperatively. These findings have tremendous implications on the prognosis of the patient, as well as the need to pursue further genetic testing, clinical monitoring and/or adjuvant therapies. In addition, important mechanisms underlying the molecular biology of HPT have been elucidated with the discovery of genetic and epigenetic alterations implicating cyclin D1 signalling (CCND1/PRAD1, CDC73/HRPT2, CDKN1B, CDKIs, ZFX), Wnt/β-catenin signalling (MEN1, APC, EZH2, CTNNB1, SFRPs) and CaSR signalling (CaSR, GNA11, AP2SI), offering promising targets for the development of new diagnostic, prognostic and predictive biomarkers.

**Pathological correlations of hyperparathyroidism include parathyroid hyperplasia, adenoma and carcinoma.**

**The primary role of the intraoperative pathological consultation is to confirm the presence of parathyroid tissue in the resected specimen and to confirm the presence of abnormal (enlarged cellular) gland.**

**Clinical and biochemical features, including intraoperative PTH levels, number, weight and size of the affected parathyroid gland(s), are crucial parameters to consider when rendering an accurate diagnosis of parathyroid proliferations.**

**The diagnosis of parathyroid carcinoma is rendered when a parathyroid neoplasm exhibits any of the followings: vascular invasion, perineural invasion, invasive growth into adjacent structures/organs, and/or metastasis.**

**The application of ancillary tools can also help to distinguish benign disease from parathyroid carcinoma. In particular, the use of parafibromin immunostain is very helpful to differentiate between parathyroid adenomas (intact nuclear and nucleolar parafibromin expression) and parathyroid carcinomas (complete loss of nuclear or nucleolar parafibromin expression).**

**Genetic and epigenetic alterations resulting in deregulation of cyclin D1, Wnt/β-catenin and CaSR signalling have been uncovered in a significant subset of patients with primary hyperparathyroidism.**

**In the absence of secondary causes of hyperparathyroidism, an underlying familial syndrome should be considered in patients with parathyroid hyperplasia and carcinoma, which may be found in respectively ~25% and ~15% of cases.**

**Handling editor Runjan Chetty**

**Contributors** OM: substantial contributions to the conception or design of the work; or the acquisition, analysis or interpretation of data for the work; final approval of the version to be published; agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. KD, KGH and OM: drafting the work or revising it critically for important intellectual content.

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