

## **Genetics of parathyroid tumours**

R. V. Thakker

From the University of Oxford, Academic Endocrine Unit, Radcliffe Department of Medicine  
OCDEM (Oxford Centre for Diabetes, Endocrinology and Metabolism), The Churchill Hospital  
Headington, Oxford, UK

### **Correspondence:**

R.V. Thakker, MD, FRS  
May Professor of Medicine  
University of Oxford  
Academic Endocrine Unit  
Radcliffe Department of Medicine  
OCDEM (Oxford Centre for Diabetes, Endocrinology and Metabolism)  
The Churchill Hospital  
Headington  
Oxford  
OX3 7LJ, UK.  
Tel: 44 (0)1865-857501  
Fax: 44 (0)1865-857502  
Email: [rajesh.thakker@ndm.ox.ac.uk](mailto:rajesh.thakker@ndm.ox.ac.uk)

## Abstract

Primary hyperparathyroidism (PHPT), due to parathyroid tumours, may occur as part of a complex syndrome or as an isolated (non-syndromic) disorder, and both forms can occur as familial (i.e. hereditary) or non-familial (i.e. sporadic) disease. Syndromic PHPT includes multiple endocrine neoplasia (MEN) types 1 to 4 (MEN1 to MEN4) and the hyperparathyroidism-jaw tumour (HPT-JT) syndrome. Syndromic and hereditary PHPT are often associated with multiple parathyroid tumours, in contrast to sporadic PHPT, in which single parathyroid adenomas are more common. In addition, parathyroid carcinomas may occur in ~15% of patients with the HPT-JT syndrome. MEN1 is caused by abnormalities of the *MEN1* gene which encodes a tumour suppressor; MEN2 and MEN3 are due to mutations of the rearranged during transfection (*RET*) proto-oncogene, which encodes a tyrosine kinase receptor; MEN4 is due to mutations of a cyclin-dependent kinase inhibitor (*CDKN1B*); and HPT-JT is due to mutations of cell division cycle 73 (*CDC73*), which encodes parafibromin. Non-syndromic PHPT, which may be hereditary and referred to as familial isolated hyperparathyroidism, may also be due to *MEN1*, *CDC73* or calcium-sensing receptor (*CASR*) mutations. In addition, ~10% of patients presenting below the age of 45 years with non-syndromic, sporadic PHPT may have *MEN1*, *CDC73* or *CASR* mutations, and overall more than 10% of patients with PHPT will have a mutation in one of 11 genes. Genetic testing is available and of value in the clinical setting, as it helps in making the correct diagnosis and planning the management of these complex disorders associated with parathyroid tumours.

**Keywords:** Primary hyperparathyroidism, hypercalcaemia, multiple endocrine neoplasia, calcium-sensing receptor, mutational analysis.

## Introduction

Parathyroid tumours are common with an annual incidence of 27–30 in 100,000 of the population, and are associated with hypercalcaemia, for example in primary hyperparathyroidism (PHPT), which affects ~3 in 1000 of the adult population and >1% of post-menopausal women [1–4]. Parathyroid tumours are also associated with significant healthcare costs. For example, use of healthcare resources has been reported to be high among patients with PHPT, with annual costs of USD 37,000 [5] and more than GBP 1000 for treatment [6]. Parathyroid tumours usually occur as a non-familial solitary endocrinopathy, also referred to as sporadic (non-syndromic) PHPT, or sometimes as a familial solitary endocrinopathy, also known as familial isolated PHPT (FIHP) (Fig. 1) [7]. However, this distinction between sporadic and familial forms in PHPT patients may be difficult, either because a family history may be absent in some sporadic cases as the parent with the disease may not have been investigated or may have died before PHPT symptoms developed or because the PHPT may be due to a *de novo* germline mutation in the patient, which would account for the lack of family history but result in an increased risk of hereditary PHPT in the children of the patient. Parathyroid tumours may also occur as part of complex endocrine syndromes, such as multiple endocrine neoplasia (MEN) [e.g. MEN1, MEN2, MEN3 (also known as MEN2b) and MEN4] (Table 1) and hyperparathyroidism-jaw tumour (HPT-JT) syndrome, which are inherited as autosomal dominant traits [7–10]. Over 10% of patients will have a hereditary form of PHPT and, to date, studies of syndromic and non-syndromic forms of PHPT (Fig. 1) have identified genetic abnormalities involving one of 11 genes (Table 2) [7, 8, 11, 12]. In addition, sporadic forms of parathyroid tumours may arise because of somatic gene abnormalities involving at least six different genes (Fig. 1) [12, 13]. The focus of this review will be the germline genetic mutations causing syndromic and non-syndromic forms of hereditary PHPT (Table 2) and their value in clinical diagnosis and management of patients.

## **Syndromic forms of primary PHPT**

The main forms of syndromic PHPT are MEN1, MEN2, MEN3, MEN4 and HPT-JT.

### *MEN1*

MEN1 is characterized by the combined occurrence of tumours of the parathyroids, pancreatic islet cells and anterior pituitary [4, 7, 14]. Parathyroid tumours, resulting in PHPT, occur in 95% of MEN1 patients, and the associated hypercalcaemia may be the first manifestation of MEN1 in about 90% of patients. Pancreatic islet cell tumours occur in 40% of MEN1 patients, with gastrinomas giving rise to multiple gastric/duodenal ulcers due to high gastric acid output; this disorder is known as the Zollinger–Ellison syndrome. Gastrinomas are the most common type of pancreatic islet cell tumours and the most important cause of morbidity and mortality in MEN1 patients. Anterior pituitary tumours occur in 30% of MEN1 patients, with prolactinomas representing the most common type. MEN1 patients may also develop other associated tumours, including adrenal cortical tumours, carcinoid tumours, lipomas and cutaneous angiofibromas and collagenomas [7]. The gene causing MEN1 is located on chromosome 11q13 (Table 1) and comprises 10 exons that encode a 610-amino acid protein, menin [7, 14]. Studies of MEN1 patients have identified over 1100 germ-line *MEN1* mutations, and the majority (>80%) of these mutations are inactivating, consistent with the role of the *MEN1* gene as a tumour suppressor [14]. In addition, the *MEN1* mutations are spread throughout the 1830 bp coding region with no evidence for clustering [14]. There do not appear to be any correlations between the *MEN1* germline mutations and the clinical manifestations of the disorder [7, 14]. Tumours from MEN1 and non-MEN1 patients have been demonstrated to

have the germ-line mutation together with a somatic loss of heterozygosity (LOH) involving chromosome 11q13 in the tumour, as expected from Knudson's model and the proposed role of the *MEN1* gene as a tumour suppressor [7, 14]. Furthermore, menin expression *in vivo*, by *MEN1* gene-replacement therapy, in anterior pituitary tumours of heterozygous knockout mice (*Men1*<sup>+/-</sup>) has also been shown to reduce tumour cell proliferation, consistent with a tumour suppressor role for menin [15]. It has been shown that menin, which is predominantly a nuclear protein in non-dividing cells, is involved in genome stability, transcription regulation, cell division and proliferation [14]. Menin also acts as a scaffold protein and may increase or decrease gene expression by epigenetic regulation via histone methylation or histone deacetylation [16].

#### *MEN2 and MEN3*

MEN2 patients present a combination of medullary thyroid carcinomas (MTCs), pheochromocytomas and parathyroid tumours [7, 9]. There are three clinical variants of MEN2, referred to as MEN2a, MEN2b (also MEN3) and MTC-only [9]. In MEN2a, the commonest variant, development of MTC is associated with parathyroid tumours in 20% of patients, and pheochromocytomas in 50% of patients, that may be bilateral [9]. In MEN2b, which represents 5% of all MEN2 cases, the occurrence of MTC and pheochromocytoma is associated with a Marfanoid habitus, medullated corneal fibres, mucosal neuromas and intestinal autonomic ganglion dysfunction leading to multiple diverticulae and megacolon [9]. Parathyroid tumours do not usually occur in MEN2b [9]. In MTC-only, MTC is the sole manifestation of the syndrome. The gene causing all three MEN2 variants is the *c-ret* proto-oncogene (*RET*) located on chromosome 10q11.2. The *RET* gene encodes a tyrosine kinase

receptor that has cadherin-like and cysteine-rich extracellular domains, and a tyrosine kinase intracellular domain [7]. Specific mutations of *RET* result in each of the three MEN2 variants. Thus, MEN2a is associated in 95% of patients with missense mutations of the cysteine-rich extracellular domain, and mutations in codon 634 (Cys→Arg) account for 85% of mutations in patients with this disorder [7]. However, sporadic non-MEN2a parathyroid adenomas do not have *RET* codon 634 mutations [17]. MTC-only is also associated with missense mutations involving the cysteine-rich extracellular domain, with most mutations in codon 618 [7]. However, MEN2b is associated in 95% of patients with mutations in codon 918 (Met→Thr) of the intracellular tyrosine kinase domain [7]. *RET* mutational analysis of codons 609, 611, 618, 634, 768 and 804 in MEN2a and MTC-only, and codon 918 in MEN2b, has been used in the diagnosis and management of patients and families with these disorders [7], with great benefit and improved outcomes for patients at risk of MTC who have undergone prophylactic thyroidectomy. Thus, 90% of young patients with a *RET* mutation who have undergone a prophylactic thyroidectomy have been reported to have no evidence of persistent or recurrent MTC at 7 years after surgery, in contrast to patients with metastatic MTC for whom 10-year survival is ~20% [7, 18, 19].

#### *MEN4*

Approximately 5–10% of patients with MEN1 do not have mutations of the *MEN1* gene [7, 14], and these patients may have mutations involving other genes. One of these genes is *CDNK1B*, which is located on chromosome 12p13 and encodes the 196-amino acid cyclin-dependent kinase inhibitor (CK1) p27<sup>kip1</sup> [10]. Studies have revealed that approximately 3% of the patients with MEN1-associated tumours, such as parathyroid adenomas, pituitary adenomas

and pancreatic NETs in association with gonadal, adrenal, renal and thyroid tumours, who do not have *MEN1* mutations, instead have *CDNK1B* mutations, and these patients are considered to have MEN4 [7, 10]. To date, eight different heterozygous loss-of-function *CDNK1B* mutations have been identified in patients with MEN1-like tumours, indicating that MEN4 is an autosomal dominant disorder [7, 10]. In addition, germline *CDNK1B* mutations may rarely be found in patients with sporadic (i.e. non-familial) forms of PHPT [20].

#### *HPT-JT syndrome*

Patients with the HPT-JT syndrome, an autosomal dominant disorder, develop parathyroid adenomas and carcinomas, in association with fibro-osseous jaw tumours [21–23], uterine tumours and renal abnormalities, such as Wilms' tumours, renal cysts, hamartomas and cortical adenomas, and papillary renal cell carcinomas [21–23]. In addition, some patients may also develop pancreatic adenocarcinomas, testicular mixed germ cell tumours with a major seminoma component, and Hurthle cell thyroid adenomas [21–23]. The gene causing HPT-JT, is the cell division cycle 73 (*CDC73*) gene, also referred to as hyperparathyroidism type 2 (*HRPT2*) [22–24]. *CDC73* is located on chromosome 1q31.2 and comprises 17 exons that encode parafibromin, a 531-amino acid protein [22]. Parafibromin is predominantly a nuclear protein, that forms part of the polymerase-associated factor 1 (PAF1) complex, which mediates key transcriptional events in histone modification, chromatin remodelling, initiation and elongation, and activates the wnt/ $\beta$ -catenin and hedgehog signalling pathways [25–28]. More than 60 heterozygous *CDC73* germline mutations have been reported in HPT-JT patients. The *CDC73* mutations are widespread throughout the 1593-bp coding region, with the majority (>80%) predicting a premature truncation and hence functional loss of parafibromin [8, 23]. A

genotype–phenotype correlation does not appear to be present [8, 21]. HPT-JT-associated tumours have LOH involving the chromosome 1q21.32 region, indicating that parafibromin may be acting as a tumour suppressor, consistent with Knudson’s two-hit hypothesis [8, 21, 22]. Indeed, the identification of germline and somatic *CDC73* mutations in HPT-JT-associated tumours is also consistent with such a tumour suppressor role for *CDC73* [21, 22, 24]. Similar germline and somatic *CDC73* mutations have also been found in 65% to 100% of sporadic parathyroid carcinomas [24], although, only 0% to 4% of sporadic parathyroid adenomas have *CDC73* mutations, indicating that *CDC73* mutations probably confer aggressive growth potential to the parathyroid cells [8, 21].

The parathyroid tumours in HPT-JT patients may occur in isolation and without any evidence of jaw or other tumours, and this may cause confusion with other inherited hypercalcaemic disorders such as MEN1, familial hypocalciuric hypercalcaemia (FHH; also known as familial benign hypercalcaemia) and FIHP [8]. HPT-JT can be distinguished from FHH, as hypercalcaemia in FHH may be detected in the early neonatal or infantile period whereas hypercalcaemia most commonly occurs during the first decade in HPT-JT [8]. In addition, HPT-JT patients, unlike those with FHH, have hypercalciuria. The distinction between HPT-JT and MEN1 patients who have only developed the usual first manifestation of hypercalcaemia (which occurs in >90% of MEN1 patients) is more difficult and is likely to depend on surgical and histological findings, and the occurrence of other characteristic lesions in each disorder [7, 8]. Thus, HPT-JT patients usually have a single parathyroid adenoma or a carcinoma, whereas MEN1 patients often have multiglandular parathyroid disease [4, 7, 8]. The distinction between FIHP and HPT-JT in the absence of jaw tumours is difficult but

important as HPT-JT patients have a higher risk of developing parathyroid carcinomas [4, 7, 8]. Identification of additional features, such as jaw tumours and renal, pancreatic, thyroid and testicular abnormalities, may help to distinguish patients with HPT-JT from those with FIHP. Indeed ossifying fibromas of the jaw are an important distinguishing feature of HPT-JT, and their occurrence may occasionally precede the development of hypercalcaemia in HPT-JT patients by several decades [8, 21]. However, it should be noted that the jaw tumours in HPT-JT are different from the brown tumours observed in some patients with PHPT and do not resolve after parathyroidectomy [8, 22].

### **Non-syndromic forms of PHPT**

#### *FIHP*

FIHP may represent an incomplete manifestation of a syndromic form of PHPT such as MEN1, HPT-JT or FHH. Moreover, FIHP in over 40 kindreds has been reported to be due to germline mutations of the *MEN1*, *CDC73* or calcium-sensing receptor (*CASR*) genes [7, 8, 23, 24, 29, 30]. The sole occurrence of parathyroid tumours in these syndromic disorders is remarkable and the mechanisms that determine the altered phenotypic expressions of these mutations remain to be elucidated. However, it is important to note that the genetic aetiology of non-syndromic FIHP in the majority of families is still unknown [14, 31].

#### *FHH and neonatal severe HPT*

FHH is an autosomal dominant disorder characterized by lifelong asymptomatic hypercalcaemia in association with an inappropriately low urinary calcium excretion [i.e. calcium clearance:creatinine clearance ratio (CCR) <0.01], and normal circulating PTH

concentrations in 80% of patients [4, 32]. Hypermagnesaemia is also typically present. Although most patients with FHH are asymptomatic, chondrocalcinosis and acute pancreatitis have occasionally been observed. Patients with FHH have been misdiagnosed as having PHPT, as 20% of FHH patients may have elevated plasma PTH concentrations [4, 7]. In addition, 20% of FHH patients may have a CCR >0.01, and therefore be indistinguishable from patients with PHPT [4, 7, 32, 33]. Moreover, low CCRs are observed in patients with PHPT who have vitamin D deficiency or renal insufficiency, or are of African-American origin [4]. It is important to distinguish FHH patients from those with PHPT, as the hypercalcaemia in FHH is generally benign and does not result in sequelae [4, 7]. Moreover, parathyroidectomy does not correct the hypercalcaemia in FHH. Mutational analysis may help to identify FHH patients and to distinguish them from those with PHPT [4, 7, 32].

FHH is genetically heterogeneous with three reported variants, FHH1, FHH2 and FHH3, with loci on chromosomes 3q21.1, 19p and 19q13, respectively (Table 2) [32, 34, 35]. FHH1 is due to heterozygous loss-of-function mutations of the calcium-sensing receptor (CaSR), which is a G-protein coupled receptor (GPCR) that signals via  $G\alpha_q$  and  $G\alpha_{11}$  [32]. Approximately two-thirds of FHH kindreds will have unique heterozygous mutations of the CaSR and expression studies of these mutations have demonstrated a loss of CaSR function whereby there is an increase in the calcium ion-dependent set-point for PTH release from the parathyroid cell [32]. FHH2 is due to loss-of-function mutations in the G-protein subunit  $\alpha_{11}$  ( $G\alpha_{11}$ ), encoded by *GNA11*. FHH2-associated  $G\alpha_{11}$  mutations decrease the sensitivity of cells expressing the CaSR, probably by impairing the release of GDP [34]. Such  $G\alpha_{11}$  loss-of-function mutations may occur in <5% of FHH patients [34]. FHH3 is due to loss-of-function mutations of the adaptor protein 2 (AP2)

sigma subunit (AP2 $\sigma$ ), encoded by *AP2S1* [35]. AP2 is a central component of clathrin-coated vesicles (CCVs) and is pivotal in clathrin-mediated endocytosis, which internalizes plasma membrane constituents such as GPCRs. AP2 is a heterotetramer of  $\alpha$ ,  $\beta$ ,  $\mu$  and  $\sigma$  subunits and links clathrin to vesicle membranes and binds to tyrosine- and dileucine-based motifs of membrane-associated cargo proteins [35]. The FHH3-associated AP2 $\sigma$  mutations, which all involve an Arg15 residue that forms key contacts with the dileucine-based motifs of CCV cargo proteins, result in a decreased sensitivity of CaSR-expressing cells to extracellular calcium and reduced CaSR endocytosis, probably through loss of interaction with a C-terminal CaSR dileucine-based motif, the disruption of which also decreased intracellular signalling [35]. Such AP2 $\sigma$  loss-of-function mutations occur in >5% of FHH patients [35].

Neonatal severe PHPT (NS-PHPT) is defined as symptomatic hypercalcaemia with skeletal manifestations of hyperparathyroidism in the first 6 months of life [7, 32]. NS-HPT patients often present in the first few days or weeks of life with failure to thrive, dehydration, hypotonia, constipation, rib cage deformities and multiple fractures due to bony undermineralization [32]. Children with NS-PHPT often have life-threatening hypercalcaemia and require urgent parathyroidectomy, which corrects the PTH-dependent hypercalcaemia and bone demineralization [7]. FHH is due to heterozygous inactivating mutations of the CaSR, and NS-HPT is often associated with inactivating homozygous CaSR mutations in the children of consanguineous parents with FHH1 [32]. However, NS-PHPT has also been observed in children in whom only one parent had clinically apparent FHH, and many other NS-PHPT cases appear to be sporadic (i.e. both parents have normal serum calcium concentrations) [32].

### *Non-familial (sporadic) PHPT due to germline mutations*

Non-familial (sporadic) forms of PHPT may be associated with germline mutations involving the *MEN1*, *CDC73*, *CASR*, *CDKIs* or *PTH* genes. Thus, ~10% of patients presenting, below the age of 45 years, with non-familial (sporadic) PHPT may have a *de novo* germline *MEN1*, *CDC73* or *CASR* mutation [4, 7, 8, 11, 30]. This has implications for the future management of these patients, in requiring screening for the occurrence of tumours associated with the specific syndrome, as well as screening for their children who may inherit the germline mutation. Moreover, >5% of patients with typically presenting PHPT, in the sixth to ninth decades of life, with solitary parathyroid adenomas and without family histories of parathyroid, MEN or other endocrine tumour syndromes may have germline mutations of genes encoding CDKIs [*CDKN1A* (p21), *CDKN2B* (p15) or *CDKN2C* (p18)], indicating that rare variants of these CDKIs may contribute to the aetiology of non-familial (sporadic) PHPT [12]. In addition, a *PTH* nonsense mutation, Arg83Stop, has been reported in a patient who had presented with hypercalcaemia and an undetectable serum concentration of intact PTH (measured using two-site sandwich assays). Following removal of the parathyroid adenoma, normocalcaemia was restored [36]. The mutation resulted in truncation of the secreted PTH after the 52<sup>nd</sup> amino acid and occurred in association with LOH of the *PTH* locus in the parathyroid adenoma [36]. These findings demonstrate that *PTH* nonsense mutations, which result in truncated forms of PTH not recognizable by standard hormone assays, may be associated with parathyroid adenoma and that endogenously produced N-terminal PTH fragments can be biologically active [36].

### **Gene testing for PHPT in clinical practice**

Genetic testing for germline mutations, which are found in ~10% of all PHPT patients, is helpful in clinical practice in several ways: (i) confirmation of the clinical diagnosis so that appropriate screening for associated tumours can be undertaken; (ii) implementation of appropriate treatment, e.g. early parathyroidectomy for patients with the HPT-JT syndrome because of the increased occurrence of parathyroid carcinomas, avoidance of minimally invasive parathyroid surgery in MEN1 patients who generally have multigland disease requiring open neck exploration, early prophylactic thyroidectomy in MEN2/MEN3 patients and avoiding surgery in FHH patients; (iii) identification of family members who may be asymptomatic but harbour the mutation and therefore require screening for tumour detection and early/appropriate treatment; and (iv) identification of the 50% of family members who do not harbour the familial germline mutation and can therefore be reassured and relieved of the anxiety burden of developing future tumours [4, 7, 19]. This latter point cannot be over-emphasized as it helps to reduce the cost to the individuals and their children, and also to the health services in not having to undertake unnecessary biochemical and radiological investigations [4, 7]. Moreover, a notable example of the improvements in patient outcome resulting from genetic testing is provided by the results of prophylactic thyroidectomy in MEN2/MEN3 patients. The 10-year survival in patients with metastatic MTC is ~20%, and prophylactic thyroidectomy to prevent MTC and its metastases is recommended in patients with a *RET* mutation [9]; such prophylactic thyroidectomy has dramatically improved outcomes in MEN2/MEN3 patients, with, as mentioned above, about 90% of young patients with a *RET* mutation who underwent prophylactic thyroidectomy having no evidence of persistent or recurrent MTC at 7 years after surgery [9, 18, 19].

### *Indications for mutational analysis in PHPT*

Indications for testing for germline mutations in PHPT patients include: (i) PHPT occurring before the age of 45 years; (ii) multigland disease; (iii) parathyroid carcinoma or atypical parathyroid adenomas (e.g. with fibrous bands or cysts); (iv) being a first-degree relative of a known mutation carrier; and (v) being an index case with two or more MEN syndrome-associated endocrine tumours [4, 7]. Genetic testing should use DNA obtained from leukocytes, salivary cells, skin cells or hair follicles (i.e. non-tumour cells), as DNA from parathyroid tumours is not clinically useful for establishing the diagnosis or staging because such tumours may contain multiple mutations. Best clinical practice for such genetic testing should include informed consent from the patient and access to genetic counsellors [4, 7]. Genetic testing should be performed at accredited centres, some of which can be contacted using the following links:

<http://www.ncbi.nlm.nih.gov/sites/GeneTests/> (with details of centres in Canada, Denmark, Greece, Israel, Japan and the USA); and <http://www.orpha.net/consor/cgi-bin/index.php> or [www.eddnal.com](http://www.eddnal.com) (with details of centres in Austria, Belgium, Denmark, Finland, France, Germany, Holland, Ireland, Italy, Norway, Portugal, Spain, Sweden, Switzerland and the UK).

### *Clinical approach to gene testing in patients with PHPT*

A clinical approach to genetic testing in a patient who has PHPT and no manifestations of MEN- or HPT-JT-associated tumours is shown in Fig. 2 [4, 7]. PHPT patients in whom there is a high suspicion of a genetic aetiology (e.g. young age at onset, multigland disease, parathyroid carcinoma or atypical parathyroid adenoma) should be offered genetic counselling and germline mutation testing of the *MEN1*, *CASR*, *AP2S1*, *GNA11*, *CDC73*, *CDKN1A*, *CDKN1B*, *CDKN2B*,

*CDKN2C*, *RET* and *PTH* genes, using DNA obtained from non-tumour cells (e.g. leukocytes) [4, 7, 11]. Such patients may have *de novo* mutations, which occur in ~10% of patients, or they may have an undisclosed family history of the disease [7]. Furthermore, PHPT may be the first manifestation of MEN1 and HPT-JT in ~90% and >95%, respectively, of patients with these disorders [7, 8, 21]. In addition, the clinical distinction between PHPT and FHH may be difficult as ~20% of FHH patients may have elevated serum PTH concentrations and a CCR of >0.01 [29, 30, 32–35], and some PHPT patients may have hypocalciuria [4]. Moreover, PHPT and FIHP may occasionally be due to *CASR* mutations [29, 30]. The identification of a germline mutation should prompt entry into appropriate periodic clinical, biochemical and radiological screening programmes, e.g. for MEN- and HPT-JT-associated tumours [4, 7]. The absence of clinical manifestations of hereditary or syndromic forms of PHPT and any genetic abnormalities within the 11 genes would indicate that the likelihood of one of the MEN syndromes, HPT-JT or FHH was low (i.e. <5%) [4, 7–9]. First-degree relatives of a PHPT patient with a germline mutation should be identified and offered genetic counselling and appropriate gene testing, and individuals who have inherited the mutation should be offered periodic screening, even if asymptomatic [4, 7–9]. First-degree relatives who have not inherited the causative mutation require no further follow-up and the anxiety associated with the development of MEN- or HPT-JT-associated tumours can be alleviated [4, 7]. For PHPT patients who present at a later age, a detailed family history should be taken to determine the presence of PHPT (i.e. FIHP), one of the MEN syndromes, HPT-JT or FHH, and appropriate clinical evaluation and gene testing should then be performed to determine the aetiology of the PHPT [4, 7–9]. Moreover, it is important to note that >5% of patients with non-familial (sporadic) PHPT presenting in the sixth to ninth decades of life with solitary parathyroid adenoma may have a germline mutation involving *CDKN1A*, *CDKN2B*

or *CDKN2C*, indicating a higher risk of developing PHPT in the first-degree relatives [12]. Thus, genetic assessment should be carried out in their first-degree relatives for the presence of the rare variants/mutations of *CDKN1A*, *CDKN2B* and *CDKN2C*, and those who have inherited the mutation should undergo periodic screening to detect the onset of hypercalcaemia to facilitate appropriate earlier treatment aimed at preventing the skeletal and renal complications of PHPT [4, 7, 11, 12].

### **Conflict of interest statement**

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**Fig. 1** Genetics of parathyroid tumours. Parathyroid tumours may arise because of germline mutations involving one of 11 genes (Table 2) resulting in familial syndromic and non-syndromic forms of primary hyperparathyroidism (PHPT), or somatic abnormalities involving the cyclin D1 (*CCND1*), retinoblastoma (*RB*), multiple endocrine neoplasia type 1 (*MEN1*), retinoblastoma interacting zinc-finger protein (*RIZ1*),  $\beta$ -catenin (*CTNNB*) and low-density lipoprotein receptor 5 (*LRP5*) genes.

**Fig. 2** Clinical approach to gene testing in a patient with primary hyperparathyroidism (PHPT). MEN, multiple endocrine neoplasia; HPT-JT, hyperparathyroid jaw-tumour syndrome; FHH, familial hypocalciuric hypercalcaemia; FIHP, familial isolated primary hyperparathyroidism;

CASR, calcium-sensing receptor; AP2S1, adaptor protein 2 sigma subunit; GNA11, G-protein alpha 11 subunit; HRPT2, hyperparathyroidism type 2; CDC73, cell division cycle 73; CDKN, cyclin-dependent kinase inhibitor; RET, rearranged during transfection proto-oncogene; PTH, parathyroid hormone. **a**PHPT presenting without manifestations of MEN-associated tumors, or tumors associated with HPT-JT. **b**Guidelines for MEN1 recommend MEN1 mutational analysis in patients with PHPT occurring before age of 30 years, and  $\approx 10\%$  of PHPT patients below the age of 45 years have been reported to have a germline mutation involving the *MEN1*, *CASR*, or *HRPT2* (*cdc73*) genes. **c**Atypical parathyroid adenoma may have cysts or fibrous bands. **d**PHPT may be the first manifestation of MEN1 and HPT-JT in  $\approx 90$  and  $\approx 95\%$  of patients, respectively, with these disorders. **e** $<5\%$  of patients presenting with non-familial (sporadic) and non-syndromic PHPT, due to solitary parathyroid adenomas in the sixth to ninth decade of life may have rare variants/ mutations of *CDKN1A*, *CDKN2B*, or *CDKN2C*. **f***CASR*, *AP2S1*, *GNA11*, *HRPT2* (*CDC73*), *CDKN1B*, and *RET* mutations are associated with FHH1, FHH3, FHH2, HPT-JT, MEN4, and MEN2, respectively. Reproduced with permission from Thakker [38].

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**Table 1** Multiple endocrine neoplasia (MEN) syndromes<sup>a</sup>

| <b>MEN1</b>                            | <b>MEN2 and MEN3</b>               | <b>MEN4</b>          |
|--|------------------------------------|----------------------|
| <i>Tumours</i>                         |                                    |                      |
| Parathyroids (95%) <sup>b</sup>        | Medullary thyroid carcinomas (99%) | Parathyroids         |
| Pancreatic islets (40%)                | Phaeochromocytomas (50%)           | Pituitary (anterior) |
| Pituitary (anterior) (30%)             | Parathyroids (20%)                 | Adrenal              |
|  |                                    | Renal                |
|  |                                    | Gonads               |
| <i>Chromosome location<sup>c</sup></i> |                                    |                      |
| 11q13                                  | 10q11.2                            | 12p13                |
| Gene product                           |                                    |                      |
| MENIN                                  | RET                                | CDKN1B (p27, KIP1)   |

<sup>a</sup>MEN is defined as the occurrence of two or more endocrine tumours in a patient.

<sup>b</sup>Percentage of MEN patients with the tumour; this has not been established for MEN4 as <20 cases have been reported.

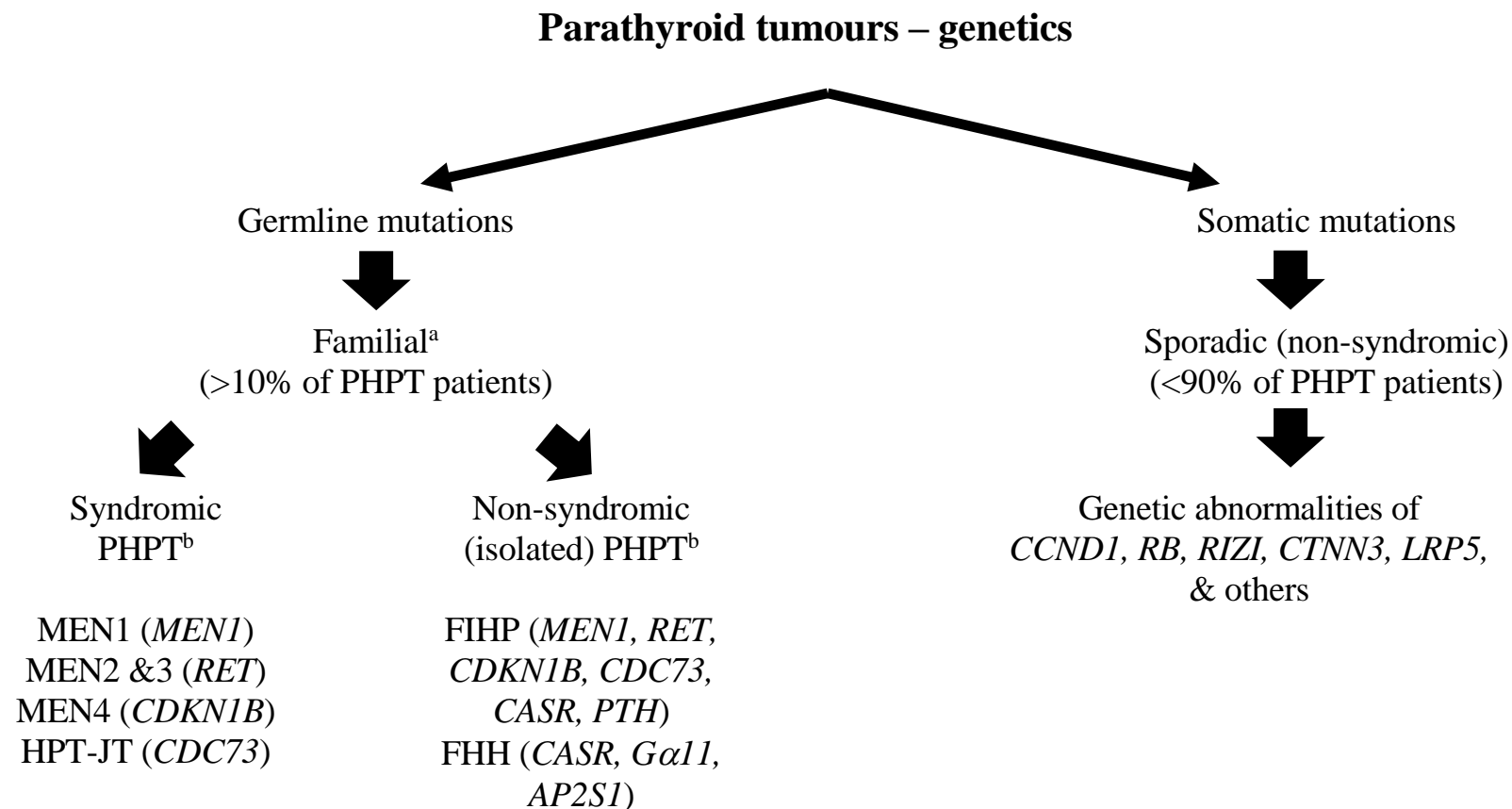
<sup>c</sup>Autosomal dominant inheritance for MEN1, MEN2, MEN3 and MEN4.

**Table 2 Genetics of hereditary primary hyperparathyroidism (PHPT) and parathyroid tumours**

| <b>Disease</b> | <b>Inheritance</b>                        | <b>Gene product</b> | <b>Chomosomal location</b> |
|----------------|---|---------------------|----------------------------|
| MEN1           | Autosomal dominant                        | MENIN               | 11q13                      |
| MEN2 and MEN3  | Autosomal dominant                        | RET                 | 10q11.2                    |
| MEN4           | Autosomal dominant                        | CDKI1B              | 12p13                      |
| HPT-JT         | Autosomal dominant                        | PARAFIBROMIN        | 1q25                       |
| FHH1           | Autosomal dominant                        | CaSR                | 3q21.1                     |
| FHH2           | Autosomal dominant                        | Gα11                | 19p13                      |
| FHH3           | Autosomal dominant                        | AP2S1               | 19q13                      |
| NS-PHPT        | Autosomal recessive<br>Autosomal dominant | CaSR                | 3q21.1                     |

MEN, multiple endocrine neoplasia; HPT-JT, hyperparathyroid jaw-tumour syndrome; FHH, familial hypocalciuric hypercalcaemia; NS-PHPT, neonatal severe primary hyperparathyroidism; AP2S1, adaptor protein 2 sigma subunit; CaSR, calcium-sensing receptor.

**Figure 1**



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<sup>a</sup>May not always appear familial because of *de novo* mutation, or parent(s) may have died before manifesting disease features.

<sup>b</sup>More than 10% of PHPT patients will have a germline mutation in one of 11 genes.

**Figure 2**

