THEMATIC REVIEW

Looking beyond the thyroid: advances in the understanding of pheochromocytoma and hyperparathyroidism phenotypes in MEN2 and of non-MEN2 familial forms

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Abstract

Over the last years, the knowledge of MEN2 and non-MEN2 familial forms of pheochromocytoma (PHEO) has increased. In MEN2, PHEO is the second most frequent disease: the penetrance and age at diagnosis depend on the mutation of RET. Given the prevalence of bilateral PHEO (50% by age 50), adrenal sparing surgery, aimed at sparing a part of the adrenal cortex to avoid adrenal insufficiency, should be systematically considered in patients with bilateral PHEO. Non-MEN2 familial forms of PHEO now include more than 20 genes: however, only small phenotypic series have been reported, suggesting that phenotypic features of isolated hereditary PHEO must be better explored, and follow-up series are needed to better understand the outcome of patients carrying mutations of these genes. The first part of this review will mainly focus on these points. In the second part, a focus will be given on MEN2 and non-MEN2 familial forms of hyperparathyroidism (HPTH). Again, the management of MEN2 HPTH should be aimed at curing the disease while preserving an optimal quality of life by a tailored parathyroidectomy. The phenotypes and outcome of MEN1-, MEN4- and HRPT2-related HPTH are briefly described, with a focus on the most recent literature data and is compared with familial hypocalciuric hypercalcemia.

Key Words

- pheochromocytoma
- hyperparathyroidism
- multiple endocrine neoplasia

Introduction

The majority of the studies published on MEN2 focused on medullary thyroid cancer (MTC), as it represents the first manifestation of the disease and can lead to a fatal outcome if undiagnosed or inappropriately treated (Wells et al. 2013). However, in familial cases with early genetic diagnosis, the guidelines recommend prophylactic
thyroidectomy leading to the absence of residual thyroid disease (Wells et al. 2015). Thus, the chronic disease risk of MEN2 is the development of pheochromocytoma (PHEO), and less frequently of hyperparathyroidism (HPTH). The aim of this review is first to detail the main characteristics and the management of MEN2 PHEO and then to define the main other etiologies of hereditary bilateral PHEO (Table 1). A similar review will be made for HPTH.

### Pheochromocytoma in MEN2 and non-MEN2 familial forms

PHEO are neuroendocrine tumors arising from adrenal medulla cells. Paragangliomas (PGL) originate from sympathetic or parasympathetic paraganglia; they have rarely been reported in MEN2 patients (less than 1% in our series of 1210 patients with MEN2) (Castinetti et al. 2014), but constitute a unique entity together with PHEO in other hereditary forms. Management of pheochromocytoma requires highly-specialized teams: pre-operative management should be aimed at normalizing blood pressure and heart rate, based on alpha-adrenergic receptor blockers, high sodium diet and fluid intake, 7–14 days before surgery, with a careful monitoring of blood pressure, as stated by the Endocrine Society guidelines (Lenders et al. 2014).

### MEN2 PHEO

#### Epidemiology and genetics

PHEO is the second most frequent disease in patients with MEN2. It is usually diagnosed in the 3rd–4th decade (Nguyen et al. 2001). Even if some reports mentioned the possibility of PHEO first, with a delayed appearance of MTC, PHEO is diagnosed concomitantly or after the diagnosis of MTC in the very large majority of cases. Imai and coworkers reported that 17% of their 144 MEN2 patients were actually diagnosed with PHEO before MTC (Imai et al. 2013), however, calcitonin measurement was not systematically performed, nor, obviously thyroidectomy, and it is thus not sure that these patients indeed had normal C-cells at the time of PHEO diagnosis.

The penetrance and age at diagnosis of PHEO in MEN2 seems to be dependent on the RET mutation. For instance, Imai and coworkers reported that the penetrance of PHEO was 52% by age 50 years in codon 634 RET mutation carriers, while it was 36% at last follow-up in patients with mutations other than codons 634 and 918 (Imai et al. 2013). In our large series based on 563 individuals presenting with MEN2 PHEO, while 60% patients with 634-RET mutations had at least one PHEO at last follow-up (median age at first diagnosis, 35 years), less than 20% of patients carrying with RET exon 10 mutations presented with PHEO at
age 35 years (Castinetti et al. 2014). A strong genotype/phenotype correlation thus exists for PHEO penetrance in MEN2 patients. However, we recently reported that the penetrance of PHEO in MEN2 was variable depending on the geographic area, with an age at first diagnosis of PHEO significantly higher in South America than that in Europe (Castinetti et al. 2017). The American Thyroid Association (ATA) guidelines recommended screening for PHEO beginning at 11 years for children in the ATA-high and highest risk (RET codons 634, 883 and 918) and 16 years in the ATA-moderate risk (all the other codons) (Wells et al. 2015). Of note, one case report described the occurrence of PHEO in an 8-year-old boy carrying a 634 RET codon mutation (Rowland et al. 2013).

Interestingly, in MEN2, patients within the same family, i.e. carrying the same mutation and theoretically exposed to the same environment, can present a different penetrance of PHEO (absence, unilateral or bilateral): this might be due to modifying genes as recently suggested to explain at least partly the difference of MTC aggressiveness in patients with MEN2A compared to MEN2B (Oczko-Wojciechowska et al. 2017). Siqueira and coworkers had already shown that RET polymorphisms had an influence on the prevalence of MEN2 PHEO (Siqueira et al. 2014). For instance, the presence of two RET variants among L769L, S836S and G691S/S904S was associated with an increased risk for early PHEO development (Siqueira et al. 2014).

The outcome of MEN2 PHEO is usually good in specialized MEN/endocrine referral centers. Thosani and coworkers reported only 2 deaths out of 84 patients, because of PHEO with hypertensive crises and autopsy diagnosis; however, the diagnostic procedure had been performed in the 1960s, and such events now appear to be very rare in the modern era of PHEO management (Thosani et al. 2013). In our series, 5 deaths (<1%) were due to PHEO, 4 because of hypertensive crises in undiagnosed patients and 1 because of diffuse PHEO metastases (Castinetti et al. 2014).

Diagnosis

Diagnosis follows the same procedure as sporadic PHEO. Of note, in our series, one-third of the patients were not symptomatic (hypertension, headaches, sweating) at the time PHEO was diagnosed (Castinetti et al. 2014). Systematic screening should thus be performed regularly even in the absence of clinical signs suggestive of PHEO. From a pathophysiological viewpoint, adrenomedullary hyperplasia precedes PHEO, and PHEO is frequently multifocal in the same adrenal gland (Korpershoek et al. 2014). Symptoms of catecholamine oversecretion can be already seen at the stage of hyperplasia. The development of PHEO in MEN2 is usually progressive, and bilateral PHEO are not always synchronous: metachronous PHEO have been reported in up to 25% cases after a mean period of 5–10 years (Thosani et al. 2013, Castinetti et al. 2014), requiring a prolonged follow-up after the first surgery. Positive diagnosis is based on increased plasma or urinary fractionated metanephrine and normetanephrine (Bravo & Tagle 2003, Eisenhofer et al. 2011). Imaging should be performed only when biochemistry becomes positive.

Imaging

Once the biochemical diagnosis is established, the PHEO needs to be localized. The commonest approach for localizing PHEO is to perform anatomical imaging studies. Although CT is a radiation-ionizing diagnostic imaging, it provides a higher resolution than MRI enabling detection of unique and multiple PHEO including those that can coexist within the same glands. Most importantly, anatomical imaging may also guides surgeons toward the most appropriate surgical strategy (total vs subtotal). Therefore, the follow-up of MEN2-related PHEO should not be delayed beyond the scheduled time for subtotal adrenalectomy (cortical-sparing surgery). Many of the MEN2 patients do not need any specific functional imaging since the tumors are almost always confined to the adrenal gland and the likelihood of metastasis is very small. Currently, several specific radiopharmaceuticals ([123I]-MIBG, [18F]-FDA, [18F]-FDOPA PET and 68Ga-DOTATAT somatostatin analogs) are available. The main advantage of [18F]-FDOPA compared to other radiopharmaceuticals is the absence of faintly uptake by normal adrenal glands. On [18F]-FDOPA PET/CT, uptake should be considered as pathological only in cases of adrenal uptake more intense than the liver with concordant enlarged gland. [18F]-FDOPA PET/CT can also detect residual MTC in patients with persistant hypercalcitoninemia (Timmers et al. 2009, Havekes et al. 2010, Taieb et al. 2012, Castinetti et al. 2015, Shamim et al. 2015).

Treatment

Adrenalectomy is the only available treatment: it should be done before thyroid surgery in case of concomitant diagnosis of MTC and PHEO. Adrenalectomy should be performed in PHEO as soon as the biology becomes positive and/or in patients regularly followed for a
size cutoff >1 cm. Bilateral adrenalectomy should only be performed when synchronous bilateral PHEO are diagnosed (Lairmore et al. 1993). Interestingly, despite the screening procedures usually leading to smaller PHEO at the time of surgery (compared with sporadic PHEO), the risk of hypertensive episodes during resection is identical: a similar pharmacological preparation predominantly with alpha blockers should thus be performed before surgery (Scholten et al. 2011b).

The question remains on the role of adrenal sparing surgery when bilateral adrenalectomy is required in such patients. We and others have reported the excellent short-term outcomes of such an approach: the idea of adrenal-sparing surgery is to take off the PHEO while maintaining 1/3rd–1/4th of the gland to allow maintenance of a normal cortisol and aldosterone function. Out of 552 patients operated, adrenal sparing surgery was performed in 114 (20.6%). Normal cortisol function was reported in 57% of patients operated for bilateral PHEO with at least 1 sparing surgery (Castinetti et al. 2014). The results were quite the same as those for Grubbs and coworkers with 58% of normal glucocorticoid function in 33 patients operated with adrenal sparing surgery (Grubbs et al. 2013). The main risk of adrenal sparing surgery is PHEO recurrence: indeed, complete adrenal medulla resection is technically impossible, and the risk of recurrence in this germinal disease is thus very high: while we had 3% risk of recurrence after 10 years of follow-up, other series reported 1–11% risk of recurrence after a mean follow-up of 6–10 years after surgery (reviewed in Castinetti et al. 2016). It is likely that this risk will increase dramatically the longer the duration of follow-up. Prolonged follow-up is thus required in these patients. As there is only a very low 1–4% risk of malignancy for MEN2 PHEO (Lee et al. 1996), we suggest that this procedure should be systematically considered in all patients with MEN2 PHEO. Of note, adrenal sparing surgery has an inherent limit due to the pathophysiology of MEN2 as PHEO are frequently multifocal in the same gland, which can make sparing surgery impossible, because of the impossibility to maintain a sufficient amount of normal cortical tissue (Korpershoek et al. 2014). Recurrence after adrenal sparing surgery will be mainly treated by total adrenalectomy, or in some very experienced centers, by another partial adrenalectomy (Brauckhoff et al. 2004).

In summary, PHEO will become the most prevalent disease of MEN2 given the fact that young familial cases are treated by prophylactic thyroidectomy. The majority of the patients will be asymptomatic at the time of diagnosis, which implies a regular biological follow-up in asymptomatic carriers. Being able to perform an early diagnosis of PHEO is mandatory to allow the surgeon to get the possibility to perform adrenal-sparing surgery, and avoid the risk of permanent post-surgical adrenal insufficiency in patients with bilateral PHEO.

**Non-MEN2 familial forms: the cluster 2 genes**

Transcriptomic studies performed over recent years established a clustered classification for PHEO/PGL (reviewed in Gimenez-Roqueplo et al. 2012, Vicha et al. 2013). Cluster 1 is characterized by activation of the hypoxia-angiogenesis pathway despite normoxia: It includes SDH- and VHL-related tumors. The hypoxia-inducible factor (HIF) is abnormally stabilized due to an impairment in the VHL-mediated degradation system and induces angiogenesis, promoting the development of PHEO and PGL (Eisenhofer et al. 2004, Barontini & Dahia 2010, Jochmanova et al. 2013, 2014). Cluster 2 includes genes which mutations lead to dysregulation of intracellular signaling pathways (PI3K/AKT, MAPK/ERK kinase), triggering tumorigenesis: RET and NF1 activate PI3K/AKT/mTOR and RAS/RAF/MAPK signaling pathways; TMEM127 enhances mTOR activity; MAX modifies the MYC-MAX-MXD1 network connected with mTOR pathway. Recently, based on mRNA analysis, Fishbein and coworkers subdivided the whole group of PHEO/PGL in 4 different entities: Wnt-altered pathway, kinase signaling pathway, pseudohypoxia pathway and what they called cortical admixture (Fishbein et al. 2017). Interestingly, germline mutations in MAX occurred in this specific subgroup and not in the Cluster 2 class (Fishbein et al. 2017). The main pathways involved in PHEO pathogenesis are depicted in Fig. 1 (data from Dahia 2014).

For this review, we will thus specifically focus on the genes classified in the cluster 2, but we will also include MAX. Cluster 1 genes will not be discussed. Of note, the differential diagnosis with MEN2 for cluster 2 is mainly theoretical as normal calcitonin level at the time of PHEO diagnosis will rule out the possibility of MEN2.

**NF1**

Neurofibromatosis type 1 (NF1 or Von Recklinghausen disease) is an autosomal dominant disease due to mutations of *NF1*, a tumor suppressor gene. NF1 is mainly characterized by neurofibromas, café au lait spots, optic pathway tumors, iris hamartomas, bony lesions and skinfold freckling (reviewed in Gutmann et al. 2017). PHEO occurs in only 0.1–10% of cases; it is however one the most...
frequent etiology of hypertension in patients with NF1 (20–50% cases). This low prevalence explains why only case reports and small series have been reported in the literature. Two small studies suggested that NF1 PHEO size was usually smaller at the time of diagnosis despite the fact that the diagnosis was made at a later age than sporadic PHEO (Shinall & Solorzano 2014, Moramarco et al. 2017). This likely explains why Kepenikian and coworkers recently showed that the majority of the patients with NF1 PHEO were not symptomatic at the time of diagnosis, emphasizing the need for systematic biological screening in patients with NF1 (Kepenekian et al. 2016). In the larger study ever published, 41 patients (out of 1415 admitted for PHEO, prevalence 2.9%) with NF1 PHEO were reported: the median age at diagnosis was 41 years (range 14–67); the median size of PHEO at the time of diagnosis was 3.4 cm (range 0.8–9.5). Bilateral pheochromocytomas were identified in 17% of the patients, while metastases were reported in 7%. The majority of patients (91%) had both metanephrine and normetanephrine secretion at diagnosis (Gruber et al. 2017).

PHEO screening in NF1 patients should be based on biology every 3 years beginning at age 10–14 years. In contrast, systematic NF1 genetic screening in patients with an apparently sporadic PHEO is not recommended, unless there are clinical signs suggestive of neurofibromatosis (Bausch et al. 2006). Optimal management of NF1 PHEO is based on adrenalectomy (Gruber et al. 2017).

**Figure 1**
Simplified overview of main genes and pathways involved in PHEO/PGL. Blue boxes, tumor suppressor genes involved in hereditary PHEO/PGL; red boxes, proto-oncogenes involved in hereditary PHEO/PGL; black arrows, simplified Krebs cycle; orange arrows, inhibition effect; dotted arrows, not well-established mechanism; green arrows, stimulating effect. AKT, RAC-alpha serine/threonine-protein kinase; ERK/MAPK1, mitogen-activated protein kinase 1; FH, fumarate hydratase; HIF1α, hypoxia-inducible factor 1 alpha subunit; HIF1β, hypoxia-inducible factor 1 beta subunit; HIF2α/EPAS1, endothelial PAS domain protein 1; IDH, isocitrate dehydrogenase; MAPK pathway, mitogen-activated protein kinase pathway; MAX, MYC-associated factor X; MDH2, malate dehydrogenase 2; mTOR, mammalian target of rapamycin; MYC, MYC proto-oncogene; NF1, neurofibromin 1; PHD/EGLN 1, 2, 3, prolyl hydroxylase domain protein/egl-9 family hypoxia-inducible factor 1, 2, 3; PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinase; RAS, rat sarcoma oncogene; RET, rearranged during transfection proto-oncogene; SDH, succinate dehydrogenase complex; TMEM127, transmembrane protein 127; VHL, Von Hippel-Lindau tumor suppressor. Data from Dahlia (2014).

Germline TMEM127 mutations were first identified as an etiology of PHEO seven years ago (Qin et al. 2010). TMEM127 mutations prevalence is estimated to be around...
2% (1.7% out of 1676 patients tested with PHEO and PGL) (Abermil et al. 2012, Bausch et al. 2017). TMEM127 is a tumor suppressor, encoding a transmembrane protein localized in several intracellular organelles (Jiang & Dahia 2011). Mutations of TMEM127 lead to a drastic decreased function of the protein with an anarchic distribution into the cytoplasm, and dysregulate mTORC1 signaling complex activation. In patients with TMEM127 mutations, the age at diagnosis of PHEO was highly variable from 20 to more than 65 years old (mean age at diagnosis, 43 years, similar to the one of patients with sporadic PHEO). One third of patients had bilateral tumors while only 1 had metastases at last follow-up. One third of the patients with TMEM127 mutations had a sporadic appearing benign PHEO. Interestingly, a history of PHEO was observed in only a quarter of familial cases, suggesting a low penetrance, even if the late age at first diagnosis of PHEO might have biased these data because of an insufficient follow-up period (Qin et al. 2010, Yao et al. 2010). Neumann and coworkers expanded the phenotype one year later, by describing the occurrence of PGL in 2 patients (1 with multiple head and neck PGL, 1 with retroperitoneal extra-adrenal tumor), leading to a prevalence of PGL of 4% in this series of 48 patients (Neumann et al. 2011). No patient had a malignant disease (Neumann et al. 2011).

Finally, the phenotype has been recently completed with multifocal PHEO and (nodular) adrenal hyperplasia (<1 cm) preceding and/or coexisting with PHEO (Toledo et al. 2015). Abermil and coworkers reported a prevalence of 0.9% (n=6 patients) of TMEM127 mutations in a large cohort of 642 unrelated patients with negative testing for classical genes of PHEO and paraganglioma (Abermil et al. 2012). The overall characteristics were comparable with a variable age at diagnosis, a sporadic presentation in half of the patients, bilaterality in half of the patients, and the lack of paraganglial tumor. In this series as in the previous one, all the 5 patients with data available on secretion were presenting with higher metanephrine and normetanephrine. Finally, age-related penetrance of TMEM127-PHEO was evaluated in 0% at 0–20 years, 3% at 21–30 years, 15% at 31–40 years, 24% at 41–50 years and 32% at 51–65 years (Toledo, JCEM, 2015). Clinical screening of TMEM127 mutation carriers should start at 20 years of age, while genetic screening of at risk individuals should be performed before this age (Toledo et al. 2015).

Of note, large TMEM127 gene deletions or duplications have never been reported in any patient (Abermil et al. 2012). Optimal management of TMEM127 PHEO is based on adrenalectomy. Interestingly, the association between pheochromocytoma/paraganglioma and renal tumors has been reported for TMEM127 mutations (Hernandez et al. 2015). Up to now, roughly 150 patients with TMEM127 mutations have been reported in the literature. For a detailed list of TMEM127 variants reported in the literature, please refer to Supplementary Table 1 (Qin et al. 2010, Neumann et al. 2011, Abermil et al. 2012, Takeich et al. 2012, Elston et al. 2013, Rattenberry et al. 2013, Curras-Freixes et al. 2015, Toledo et al. 2015, Patocs et al. 2016, Bausch et al. 2017).

### MYC associated factor X (MAX)

MAX mutations were first identified as a cause of PHEO 6 years ago (Comino-Mendez et al. 2011). MAX, a tumor suppressor gene, is a component of the MYC-MAX-MXD1 network of basic helix-loop-helix leucine zipper transcription factors, regulating cell proliferation, differentiation and apoptosis. It is likely that MAX acts as a negative regulator of the network: germline mutations of MAX disable its repressing activity and lead to MYC dysregulation and cancer predisposition (Cascon & Robledo 2012). Twelve cases were reported by Comindo-Mendez and coworkers: age at diagnosis ranged from 17 to 47 years old; the majority of the patients were presenting with bilateral PHEO at diagnosis, and 3 patients had metastatic PHEO at last follow-up (Comino-Mendez et al. 2011). These characteristics were confirmed by a second prevalence study in a series of 1694 patients with PHEO and no mutation in other susceptibility genes: 16 MAX mutations were identified in 23 index patients (prevalence, 1.1%). The majority of them had bilateral PHEO, and 16% had additional thoraco-abdominal PGL. Two patients were metastatic at last follow-up. The secretion profile was mainly high secretion of normetanephrine with normal or moderately increased metanephrine. Of note, 2 patients presented a renal carcinoma and a renal oncocytoma (Burnichon et al. 2012). This finding was recently confirmed in the first study reporting a large genomic deletion of MAX in 3 siblings presenting with bilateral PHEO (n=2) and a renal oncocytoma (n=1). This could suggest that MAX also acts as a suppressor gene for renal oncocytomas (Korpershoek et al. 2016), and change the way to follow such patients (with a systematic screening for renal cancers on a long-term basis?). Up to now, roughly 60 patients with MAX mutations have been reported in the literature. For a detailed list of cases of MAX variants reported in the literature, please refer to Supplementary Table 2 (Comino-Mendez et al. 2011, 2015, Burnichon et al. 2012, Peczkowska et al. 2013, Welander et al. 2014, Bausch et al. 2017, Romanet et al. 2017).
Hyperparathyroidism in MEN2 and non-MEN2 familial forms

Primary HPTH is a rather frequent disease, with an incidence of 1 per 1000, including 5–10% of hereditary etiology. These genetic etiologies include isolated primary hyperparathyroidism (such as in familial hypocalciuric hypercalcemia) and syndromic HPTH, among which RET mutations. Familial isolated HPTH (FIHP; OMIM 145000) is defined as autosomal dominant hereditary HPTH without any other associated endocrinopathies. In the majority of families, the genetics background remains unknown, but FIHP has been reported to be associated with mutations in the MEN1, CaSR, CDC73 and more recently GCM2 genes (Table 2) (based on Miedlich et al. 2003, Thakker et al. 2012, Iacobone et al. 2015, Guan et al. 2016, Vargas-Poussou et al. 2016).

MEN2 hyperparathyroidism

Epidemiology and genetics

HPTH penetrance is lower than MTC and PHEO in MEN2. The prevalence is estimated to be 20% in MEN2A cases.

Table 2 Etiologies of hereditary HPTH and phenotypic descriptions.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Syndromic hereditary HPTH</th>
<th>Multiple endocrine neoplasia type 1</th>
<th>MEN1 tumor suppressor gene</th>
<th>CDC73 tumor suppressor gene</th>
<th>Multiple endocrine neoplasia type 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene</td>
<td></td>
<td></td>
<td>Primary HPTH (90%),</td>
<td>Primary HPTH (95%),</td>
<td>CaSR tumor suppressor gene</td>
</tr>
<tr>
<td>Gene function</td>
<td></td>
<td></td>
<td>Neuroendocrine duodenopancreatic tumor (30–70%),</td>
<td>parathyroid carcinoma (21%), fibro-osseous tumour (30%), renal tumour (13%), uterine tumour (57% of females)</td>
<td>no identified gene</td>
</tr>
<tr>
<td>Chromosomal locus</td>
<td></td>
<td></td>
<td>11q13.1 AD</td>
<td>1q31.2 AD</td>
<td>12p13.1 AD</td>
</tr>
<tr>
<td>Inheritance</td>
<td></td>
<td></td>
<td>AD</td>
<td>AD</td>
<td>AD</td>
</tr>
<tr>
<td>Phenotype</td>
<td></td>
<td></td>
<td>Primary isolated HPTH (FIPH)</td>
<td>CaSR signaling pathway</td>
<td>Primary isolated HPTH</td>
</tr>
<tr>
<td>Prevalence</td>
<td>1/30,000</td>
<td>Unknown</td>
<td>18% of FIPH</td>
<td>7% of FIPH</td>
<td>Unknown</td>
</tr>
<tr>
<td>Median age of HPTH</td>
<td>20–25 years</td>
<td>27 years</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>Disease</td>
<td></td>
<td></td>
<td>Neonatal severe HPTH</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>Gene</td>
<td></td>
<td></td>
<td>FHH1 CaSR</td>
<td>FHH2 GNA11 CaSR signaling pathway</td>
<td>FHH3 AP2S1 CaSR signaling pathway</td>
</tr>
<tr>
<td>Gene function</td>
<td></td>
<td></td>
<td>CaSR signaling pathway</td>
<td>CaSR signaling pathway</td>
<td>CaSR signaling pathway</td>
</tr>
<tr>
<td>Chromosomal locus</td>
<td></td>
<td></td>
<td>3q21.1 AR</td>
<td>3q21.1 AD</td>
<td>19q13.3 AD</td>
</tr>
<tr>
<td>Inheritance</td>
<td></td>
<td></td>
<td>AD</td>
<td>AD</td>
<td>AD</td>
</tr>
<tr>
<td>Phenotype</td>
<td></td>
<td></td>
<td>Severe hypercalcemia, lethal without parathyroidectomy</td>
<td>Longlife hypercalcemia, relative hypocalciuremia, normal or mild elevated PTH</td>
<td>Longlife hypercalcemia, relative hypocalciuremia, normal or mild elevated PTH</td>
</tr>
<tr>
<td>Prevalence</td>
<td>Unknown</td>
<td>Unknown</td>
<td>1/10,000 to 1/100,000</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>Median age of HPTH</td>
<td>Neonatal</td>
<td>Unknown</td>
<td></td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

AD, autosomal dominant; AR, autosomal recessive; FHH, Familial hypocalciuric hypercalcemia; HPTH, hyperparathyroidism.
(while it is always absent in MEN2); some series even reported a much lower prevalence of 5% in patients with RET mutations (Frank-Raue et al. 2011, Elisei et al. 2012, Machens et al. 2013). In MEN2A, hyperparathyroidism seems to be more frequently associated with 634-RET mutations than other codons (Kraimps et al. 1996, Karga et al. 1998, Raue & Frank-Raue 2009, Valdes et al. 2015). Twenty years ago, Schuffenecker and coworkers had reported that the prevalence of hyperparathyroidism did not differ between patients with C634R or other 634 codon mutations; however, there was a wide intervariability among families, with a prevalence varying from 9 to 40% (again suggesting the involvement of modifying or environmental factors) (Schuffenecker et al. 1998).

Parathyroid hyperplasia precedes the development of adenoma formation, but the hyperplastic stage can lead to hypercalcemia (Mete & Asa 2013). Moreover, hyperplasia and adenoma can coexist in several glands at the time of diagnosis, in favor of an asynchronous development of the parathyroid disease in MEN2. This means that at a given time point, some glands can still be normal, and recurrence will happen after a more or less prolonged period of time after partial parathyroidectomy. In contrast, parathyroid carcinoma has never been reported in MEN2. In sporadic hyperparathyroidism, patients are usually older, with a mean age at diagnosis close to 60 years, and they have only 1 pathologic parathyroid gland. In contrast, the mean age at diagnosis of hyperparathyroidism in MEN2 is usually close to 40 years (Kraimps et al. 1996, Schuffenecker et al. 1998, Machens et al. 2013).

MTC precedes hyperparathyroidism, even if some rare cases depicted early diagnosis of hyperparathyroidism (Mian et al. 2009, Magalhaes et al. 2011). MEN2 diagnosis is thus rarely made after an isolated diagnosis of primary hyperparathyroidism. The majority of the patients are asymptomatic at the time of diagnosis (Carling & Udelsman 2005), emphasizing the need for a systematic yearly calcium work-up in patients with MEN2, as stated by the ATA guidelines.

Diagnosis and screening
The ATA recommends to begin screening by age 8 years in patients with MEN2A, and to screen on a yearly basis for biology suggesting hyperparathyroidism in asymptomatic carriers whatever the codon (Wells et al. 2015). The diagnosis procedure is the same as the one performed in sporadic hyperparathyroidism, with at least calcium level and albumin and then PTH, phosphate and urinary calcium (Elisei et al. 2012). In case of positive diagnosis, imaging can be made to help the surgeon, even if multiglandular disease is present in the majority of cases, and bilateral neck compartments exploration is usually mandatory.

Imaging
When HPTH is diagnosed in a MTC patient, minimally invasive parathyroidectomy approach is not a recommended surgical approach for HPTH since bilateral cervicotomy is required for total thyroidectomy. In this situation, neck ultrasound enables preoperative staging of MTC and localization of parathyroid lesions. The role of radionuclide imaging is more limited since ectopic or supernumerary glands are very rare and most of the lesions can be removed via the cervical route. By contrast, when HPTH is diagnosed after thyroidectomy for MTC, preoperative localizing studies are needed for directing focused approaches (concordance between neck ultrasound and PS for the same abnormality). The optimal protocol should use the $^{99m}$Tc-MIBI/$^{123}$I subtraction protocol with pinhole acquisition (Hindie et al. 2009, 2015). The use of 4-dimensional computed tomography (4D-CT) for parathyroid imaging has been reported but increases radiation exposure to the patient (Philip et al. 2008). $^{18F}$-fluorocholine PET/CT was found to be very sensitive and specific in patients with sporadic primary HPTH or renal HPTH and would need to be evaluated in the setting of MEN2 patients, especially in cases with recurrent HPTH and negative or discordant imaging findings.

Management
In MEN2, management raises the question of the extent of parathyroidectomy, which should be performed when HPTH is diagnosed in the preoperative workup of MTC: total parathyroidectomy, total parathyroidectomy with autotransplantation or selective parathyroidectomy with removal of macroscopically abnormal glands (Herfarth et al. 1996, Yoshida et al. 2009, Scholten et al. 2011a). Total parathyroidectomy without autotransplantation will lead to permanent hypoparathyroidism, a condition not always easy to handle. Recently, Moley and coworkers reported their experience of the management of parathyroid glands during preventive thyroidectomy in patients with MEN2 (Moley et al. 2015). They did not notice any difference between preventive thyroidectomy, central neck dissection, total parathyroidectomy and autotransplantation to the forearm or to the neck,
compared to preventive thyroidectomy attempting to preserve the parathyroid glands in situ with an intact vascular pedicle (autotransplantation only if the parathyroid did not seem viable or could not be preserved intact): permanent hypoparathyroidism was not significantly different between both groups (6% vs 1%, \( P=0.1 \)) (Moley et al. 2015). Of note, it is not recommended currently to perform systematic parathyroidectomy in patients with MEN2 requiring thyroid surgery for MTC (either prophylactic or not), and still normal PTH and calcium level. In contrast, when HPTH is diagnosed after thyroidectomy, a tailored parathyroidectomy should be performed based, as previously stated, on an exhaustive imaging workup.

**Non-MEN2 hyperparathyroidism**

**Multiple endocrine neoplasia type 1 (MEN1) hyperparathyroidism**

One objective of this review is to discuss the novelties of non-MEN2 familial forms of hyperparathyroidism. We thus will not discuss in detail MEN1, but will specifically focus on studies published during the last 3–4 years. Hyperparathyroidism is the earliest and most common feature of MEN1, with a median age at diagnosis of 20–25 years, and a prevalence of almost 100% of cases by age 50–60 years. An epidemiological overview of MEN1 patients diagnosed before age 21 years showed that only 56% of the patients presented with primary hyperparathyroidism as the initial occurring disease in MEN1. The first symptoms appeared before 10 years in 14% of cases, and before 5 in 3% of cases. This emphasizes the need for genetic testing when any of the disease listed as potentially due to MEN1 occurs in a young patient (Goudet et al. 2015). MEN1 genetic screening should be performed in any patient with primary hyperparathyroidism manifestations occurring before age 30 years or in any patient with multiglandular disease whatever the age (Thakker et al. 2012, Lassen et al. 2014).

The natural history of hyperparathyroidism in MEN1 is similar to the one reported for MEN2, with an asynchronous disease (adenoma, hyperplasia on one or several glands) (Mete & Asa 2013). Interestingly, parathyroid carcinoma, though rare, has been reported in patients with MEN1, without certainty that the carcinoma was specific to the menin status. In a series of 348 patients with MEN1 followed in a single tertiary care center (the Mayo Clinic), a prevalence of 0.28% (1 case) of parathyroid carcinoma was reported (Singh Ospina et al. 2014). In parallel, the MD Anderson Cancer Center also identified 2 patients in a series of 291, leading to a prevalence of 0.8%. This may not be different from the prevalence of parathyroid carcinoma in patients with sporadic primary hyperparathyroidism (0.74%) (Christakis et al. 2016). The pathological diagnosis of parathyroid carcinoma is usually difficult, and based on invasion of the thyroid, the laryngeal nerve or other neck structures. Treatment is usually based on parathyroidectomy. The main risk is biological recurrence, rather than systemic metastasis, requiring further surgery or the use of cinacalcet (Sensipar), which is marketed in this specific indication (Singh Ospina et al. 2014, Christakis et al. 2016).

Initial surgery mostly relies on subtotal rather than total parathyroidectomy with autotransplantation. In recurrent cases, parathyroid surgery should be repeated after a complete imaging workup to identify the cause (hyperplasia of the parathyroid remnant, supranumerary/ectopic glands) and allow to perform a tailored approach in experienced surgical hands, repeat surgery usually leads to normal calcium levels (when a piece of parathyroid gland is maintained) or to hypoparathyroidism. When surgery is impossible, an alternate medical treatment is possible. Giusti and coworkers recently reported their experience with the use of cinacalcet therapy in patients with MEN1 (Giusti et al. 2016). Cinacalcet is able to bind the calcium sensor receptor and increases its sensitivity to extracellular calcium: this leads to decreased levels of PTH and calcium. In this 12-month multicenter prospective, open-label, non-comparative trial performed in 33 patients with MEN1 (22 with contra-indication or refusal to surgery as a first line treatment, 11 with contra-indications to surgery in a context of recurrent hyperparathyroidism), cinacalcet was able to normalize calcium level in 89% of the patients at the end of the study, with 30 or 60 mg cinacalcet daily. Five patients were excluded because of bad tolerance to the drug, not allowing to adjust the dose of cinacalcet. Of note, no significant change was observed in terms of PTH or urinary calcium at the end of the study (Giusti et al. 2016).

**Multiple endocrine neoplasia type 4 (MEN4)**

MEN1-like syndrome occurs in 5–10% patients without *menin* mutations. A subgroup of these patients (roughly 2%) present with *CDKN1B* (p27) mutations. Identification of p27 mutations were based on a naturally occurring MEN1 rat model (MENX) presenting with an 8bp homozygous frameshift insertion leading to a premature
stop codon in p27 (Pellegata et al. 2006). A small number of patients with heterozygous p27 mutations have been reported since then, characterized by a wide variability in all other diseases classically occurring in MEN1. Primary hyperparathyroidism, in contrast, was present in all published cases, with an identical multiglandular involvement (Agarwal et al. 2009). Interestingly, the V109G p27 variant has been reported as responsible for modifying the natural history of parathyroid involvement in MEN1, based on a cohort of 100 patients paired with 855 controls: V109G variant was associated with a more frequent multiglandular involvement at diagnosis (3–4 vs 1–2 glandular disease) (Longuini et al. 2014).

Hyperparathyroidism-Jaw tumor (HPT-JT) syndrome

HPT-JT is defined by the association of primary hyperparathyroidism, due to a single or multiple parathyroid adenoma or a parathyroid carcinoma (30% cases), and a fibro-osseous jaw tumor (30% cases). Uterine or kidney tumors have also been described as associated with this syndrome. Of note, primary hyperparathyroidism can be isolated at diagnosis, and the penetrance of other diseases is incomplete. Differentiating between MEN1 and HPT-JT can thus be challenging in case of isolated hyperparathyroidism and should lead to genetic testing of both menin and CDC73. HPT-JT is due to mutations of CDC73 (HRPT2) tumor suppressor gene, transmitted as an autosomal dominant trait (Carpten et al. 2002). CDC73 mutations lead to the loss of expression of parafibromin, a nuclear protein involved in chromatin remodeling and histone modification (reviewed in Thakker 2016). Bricaire and coworkers reported the characteristics of 20 index patients with a germinal HRPT2 abnormality; mean age at diagnosis was close to the one reported for MEN1 (23 years), but calcium level at diagnosis was higher (mean, 3.19 mmol/L) (Bricaire et al. 2013). Interestingly, a large deletion of HRPT2 was observed in a third of the cohort patients (Bricaire et al. 2013). Management of HPTH in HPT-JT is based on parathyroidectomy with bilateral neck compartments exploration, given the possible multiglandular and potential malignant nature of the disease. In contrast with sporadic primary hyperparathyroidism, follow-up should be prolonged on a long-term basis to detect recurrence (observed in up to 80% of operated patients) requiring a more aggressive parathyroid surgery (Sarquis et al. 2008). Interestingly, somatic HRPT2 mutations have also been detected in sporadic parathyroid carcinomas, arguing for a role of CDC73 mutations in the overall prognosis of parathyroid disease (Shattuck et al. 2003).

The peculiar case of familial hypocalciuric hypercalcemia

Type 1, 2 and 3 familial hypocalciuric hypercalcemia (FHH) are due to an abnormal inactivation of the calcium sensor receptor signaling pathway. Biological workup usually shows hypercalcemia, unsuppressed PTH level (mostly normal) and not-increased calcitriol (low or normal). Basically, any biological phenotype can be seen (Vargas-Poussou et al. 2016). Calcium sensor receptor gene is located in 3q21.1, encoding for the calcium sensor receptor, a transmembrane G protein-coupled receptor. FHH type 1 is due to inactivating mutations of CASR, FHH2 to inactivating GNA11 mutations and FHH3 to mutations of AP2S1, involved in calcium sensor endocytosis. FHH usually leads to parathyroid hyperplasia, even if some operated patients actually presented a true parathyroid adenoma.

Perspectives and conclusions

MEN2 is now a well-characterized disease; while original descriptions were almost exclusively focusing on MTC, reports published over the last 10 years provide more insights into the pathophysiology, diagnosis and management of PHEO, and to a lower extent, HPTH. Both diseases are characterized by a usually benign tumor profile preceded by a hyperplasic stage. Management of such tumors should thus be aimed at curing the disease while preserving an optimal quality of life (partial parathyroidectomy, partial adrenal surgery for instance, should be systematically considered in such patients). Future studies should be aimed at better exploring the phenotypes (with the help of large-scale international networks) and understanding the wide variability presented by the patients in terms of age at diagnosis, asynchronous disease, as this individualized approach will help tailoring the treatment for each patient.

In non-MEN2 familial forms of PHEO and HPTH, the genetic spectrum has drastically changed over the last 10 years, with an increasing rate of new identified genetic etiologies (especially in PHEO). Interestingly, some of these etiologies have also been linked to renal carcinogenesis, and this will be something to take into account when following patients on a long-term basis. New genes are to be identified as recently shown by Fishbein and coworkers: for instance, fusion genes involving MAML3, that have been reported in other tumor types, might play a role in the pathogenesis of hereditary PHEO (Fishbein et al. 2017). In the future, next-generation sequencing approaches will likely lead to focus on different issues,
by questioning about the pathogenicity of variants of unknown significance. Moreover, for new genes such as \textit{MAX} and \textit{TMEM127}, only little is known about the natural history of PHEO and extra-adrenal features. Large-scale follow-up studies will thus be necessary to adapt the management. New genes are also to be identified as recently shown by Fishbein and coworkers: for instance, fusion genes involving \textit{MAML3}, that have been reported in other tumor types, might play a role in the pathogenesis of hereditary PHEO (\textit{Fishbein, Cancer cell}).

Despite the fact that MEN1 has been identified decades ago, and HRPT2 roughly 15 years ago, there is still requirement for further studies, with unresolved questions similar to what we reported for MEN2 (Castinetti et al. 2014): little is known about the factors explaining the wide intra-familial variability of patients carrying with such mutations: the hypotheses of modifying genes/factors or epigenetics should thus pave the way for future research.

\section*{Supplementary data}
This is linked to the online version of the paper at \url{https://doi.org/10.1530/ERC-17-0266}.

\section*{Declaration of interest}
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