CLINICAL STUDY

Pheochromocytoma in multiple endocrine neoplasia type 2: a prospective study

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Abstract

Objective: The aim of this prospective study is to update our knowledge of the chronology of pheochromocytoma occurrence in multiple endocrine neoplasia type 2 (MEN 2), and to better manage MEN 2 patients after the genetic diagnosis.

Design: Eighty-seven non-index gene carrier MEN 2 patients were included in this prospective study: 84 patients with MEN 2A (from 52 families) and 3 with MEN 2B (from 3 families).

Methods: Medullary thyroid carcinoma (MTC) was diagnosed by measuring plasma calcitonin in basal conditions or after pentagastrin stimulation. The search for pheochromocytoma consisted of clinical evaluation, 24 h determination of urinary catecholamines and adrenal imaging. The mean age at genetic diagnosis of MEN 2 was 14.0 ± 7.0 years, the mean duration for the follow-up was 7.6 ± 2.8 years.

Results: All 87 patients had a MTC detected at the same time as the genetic diagnosis was made. Urinary catecholamine measurements led to the diagnosis of pheochromocytoma and a combination of imaging techniques enabled the correct localization of both unilateral or bilateral adrenal involvement. Pheochromocytoma was detected simultaneously with MTC in only seven patients, and seven others were detected throughout the follow-up. Of the 14 patients with pheochromocytoma, 11 had bilateral involvement: nine were initially bilateral and two became so during follow-up.

Conclusion: This study demonstrates that in MEN 2, MTC is the lesion which appears earliest. Pheochromocytoma develops later during the evolution of the disease, and necessitates regular clinical and biological monitoring throughout follow-up. Determination of urinary and/or plasma catecholamines and metanephrines should be performed to detect pheochromocytoma. Imaging techniques lead to the detection of both unilateral and bilateral pheochromocytoma, thus making video-assisted laparoscopic adrenalectomy possible.

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Introduction

Pheochromocytoma occurs in 15–20% of autosomal dominant inherited disorders (1): multiple endocrine neoplasia type 2 (MEN 2), von Hippel–Lindau disease and neurofibromatosis type 1. Of these three familial pathologies, pheochromocytoma associated with MEN 2 is the most frequent (1/25 000) (2). MEN 2A (60% of MEN 2 cases) associates medullary thyroid carcinoma (MTC) (100% of cases) with pheochromocytoma in 50% of cases and with primary hyperparathyroidism (pHPT) in 5–20% of cases. MEN 2B (5% of MEN 2 cases) is characterized by the association of MTC (100%

of cases) with pheochromocytoma (about 50% of the cases), as well as a phenotype including skeletal abnormalities suggestive of Marfan syndrome and the presence of multiple mucosal neuroma; no pHPT is found in MEN 2B. Familial MTC (FMTC) represents only 35% of MEN 2 and is characterized by the absence of other associations throughout the entire follow-up.

MEN 2 subtypes are unified by the identification of various proto-oncogene *RET* mutations which are responsible for this inherited disease (3–6).

Data obtained by retrospective studies (7–11) have shown that pheochromocytoma is diagnosed between 30 and 40 years of age, i.e., in the same age group as

the diagnosis of MTC; it is simultaneously diagnosed with MTC in 35–73% of cases and it revealed the MEN 2 in 9–25% of cases. The MEN 2 prognosis is linked to the carcinological evolution of MTC, but these retrospective studies have shown that complications related to pheochromocytoma were responsible for 64% of deaths in MEN 2A, mainly by acute accidents (7, 9).

The aim of this study is to update our knowledge of pheochromocytoma in MEN 2A and MEN 2B kindred, diagnosed through genetic familial screening, in order to improve the management of this disease.

Patients and methods

Patients

All the patients and their families included in this study are registered in the national data base of the French calcitonin tumors study group (GETC) (12) which is authorized by the French National Ethical Committee (CNIL). Written consent was obtained before inclusion of data on the data base.

We considered only non-index patients, descendants of index cases. The prospective protocol was initiated in 1989 with inclusions and follow-up until May 1999. MEN 2A or 2B was diagnosed by systematic DNA linkage analysis between 1989 and 1994, then by the identification by sequence analysis of mutations of the proto-oncogene *RET* for the whole population since 1994 (13).

Methods

After the genetic diagnosis of MEN 2, we performed initial clinical and biological monitoring, which was repeated every year throughout the entire follow-up period, in order to diagnose the different MEN 2 endocrinopathies.

Clinical evaluation Clinical evaluation consisted mainly of the detection of the classical symptoms of catecholamine excess (hypertension, postural hypotension, palpitations, headaches and excessive sweating).

Biology Pathology of thyroid C cells (C cell hyperplasia, micro or macro MTC) was investigated by measuring plasma calcitonin in basal conditions or after intravenous injection of pentagastrin if basal calcitonin was normal. In all the GETC centers the plasma calcitonin was determined using the same immunoradiometric assay (Elsa-CT, Cis-bioindustries, France) including monoclonal antibodies that recognize the 11–17 and 24–32 regions of the calcitonin molecule. There is no cross-reaction with serum pro-calcitonin, and this method is considered specific for mature calcitonin monomers. The sensitivity of the assay was 2 pg/ml, the intra- and interassay coefficients of variation were 6.7 and 7.9% respectively for values between 30 and

100 pg/ml. Basal calcitonin was considered normal if values were under 10 pg/ml (14, 15) and if there was no increase after pentagastrin injection (according to the protocol used in all the GETC centers for the pentagastrin test) (16, 17).

Hyperparathyroidism was evaluated by calcemia correlated to plasma 1–84 parathyroid hormone immunometric assays (which differ from center to center).

Pheochromocytoma was biologically diagnosed by the demonstration of an increase in the 24 h urinary excretion rate of catecholamines (total, epinephrine and norepinephrine, both unconjugated and methoxy metabolites) using high performance liquid chromatography with electrochemical detection. Results were classed as normal or pathologic according to the normal ranges given by each center.

Imaging We performed a computed tomography (CT) scan or magnetic resonance imaging (MRI) in patients with clinical symptoms of pheochromocytoma and/or with elevated urinary catecholamines. For patients without clinical symptoms or biological abnormalities, CT scans or MRI were systematically performed every 5 years from the age of 10. Metaiodobenzyl guanidine scintigraphy (MIBG) using ^{131}I or ^{123}I , depending on age and center, was performed before adrenalectomy in 11 of the 14 patients diagnosed with pheochromocytoma.

Results

The study population consisted of 87 patients: 84 with MEN 2A (from 52 families) and three with MEN 2B (from 3 families). The mean age at genetic diagnosis of MEN 2A or 2B was 14.0 ± 7.0 years (range, 0.8–29 years). The mean duration for follow-up was 7.6 ± 2.8 years (range, 1.5–10 years). In this population, all the patients had a MTC. Only 14 (16.1%, 12 MEN 2A and 2 MEN 2B) developed pheochromocytoma. Four of them (4.6%) had hyperparathyroidism (one at 18, one at 28 and two at 29 years old; patients 12, 5, 2 and 10 respectively), all were biologically diagnosed at the same time as genetic diagnosis. The mean age at pheochromocytoma diagnosis was 23.2 ± 6 years old (range, 12–30 years of age). All patients were still alive at the time of writing.

RET mutations in MEN 2 patients

In our series of MEN 2A families (see Table 1) most of the *RET* mutations reported in previous studies were found: exon 11 (codon 634), and also with lower frequency in exon 10 (codon 609, 611, 618 and 620); all of the 12 MEN 2A patients with pheochromocytoma had a *RET* mutation in exon 11 at codon 634. They presented tgc to cgc (7 patients) and tgc to tac (5 patients) nucleotide mutations at codon 634. In the three MEN 2B patients, *RET* mutations were found in

Table 1 Proto-oncogene *RET* mutations found in the 87 non-index MEN 2 gene carriers.

Exon	Codon	Nucleotide mutations	No. families (n)	Total patients (n)	No. patients with pheochromocytoma (n)
11	634 (tgc)	tac	22	33	5
		cgc	18	28	7
		ggc	1	4	0
		tcc	3	4	0
		ttc	1	7	0
		tgg	1	1	0
		Total	46	74	12
10	609 (tgc)	tac	1	1	0
	611 (tgc)	tac	1	1	0
	618 (tgc)	cgc	2	2	0
	620 (tgc)	ttc	1	3	0
		cgc	1	3	0
		Total	2	6	0
16	918 (atg)	acg	3	3	2
Total		—	55	87	14

exon 16 at codon 918 with an acg nucleotide mutation.

Clinical and biological diagnosis of pheochromocytoma

Five of the 14 patients with pheochromocytoma (Table 2) were asymptomatic. None of the symptomatic patients presented the classical symptomatic triad (headache, sweating and palpitations) associated with hypertension. Total urinary catecholamines were highly elevated in 11 (78.5%) and total urinary methoxyamines in 13 of the 14 patients (92.8%) with pheochromocytoma. In all cases, the combination of the two biological indicators for abnormal catecholamine secretion led to the diagnosis of pheochromocytoma.

Morphological diagnosis (Table 2)

Imaging was performed in all 87 patients during the follow-up period. CT scans were performed in 12 of the 14 patients with pheochromocytoma. Scanning correctly identified uni- or bilateral adrenal involvement in 9 of 12 patients but visualized only unilateral adrenal mass in three with bilateral involvement. Five MRI and 11 MIBG scintigraphies were performed before adrenal surgery: scintigraphy correctly identified adrenal involvement except in one case (patient 10, Table 2). In 92.8% of patients, the combination of CT scanning or MRI and MIBG scintigraphy resulted in the correct localization of both unilateral and bilateral pheochromocytoma.

Chronology of pheochromocytoma versus MTC

In all 87 patients, MTC was detected at the same time as the genetic diagnosis, even in patients under the age

of 10. However, pheochromocytoma was only detected simultaneously with MTC in seven of the 87 patients (Fig. 1). In our series, the youngest age at which pheochromocytoma was detected simultaneously with MTC was 12 years: this was an ectopic pheochromocytoma in MEN 2B. Throughout follow-up, seven other pheochromocytoma (8%) were diagnosed after MTC (Fig. 2) at a time interval of 1.5–8 years between MTC and pheochromocytoma diagnosis.

Of the 14 patients with pheochromocytoma, 11 (78.6%) had a bilateral involvement: nine were initially bilateral and two became so 4.5 and 6 years later (Fig. 2). Only three pheochromocytoma remained unilateral throughout the follow-up period.

Type of surgery and histological data

All the patients who underwent surgery had positive biochemical tests and imaging. Twenty-five total adrenalectomies were performed. Except in one case, a bilateral adrenalectomy was performed only if a bilateral pheochromocytoma was shown by imaging. Both open (in 7 patients) and video-assisted laparoscopic surgery (in 7 patients) were used for bilateral or unilateral adrenalectomy, depending on the surgical team and the time period. One pheochromocytoma was ectopic, localized in the adrenal area (patient 14, Table 2). Adrenal medullar hyperplasia was associated with adenoma in 10 of the 25 removed pheochromocytoma; nine of these were multicentric. The mean diameter of pheochromocytoma was 21 ± 16 mm (range, 7 mm (patient 6) to 40 mm (patient 12)). There were no perioperative complications and no malignant pheochromocytoma in our series. After surgery, none of the patients who underwent bilateral adrenalectomy and

Table 2 Clinical, biological and imaging characteristics of patients with pheochromocytoma.

Patient no.	Age at pheochromocytoma diagnosis	Symptoms	Urinary catecholamines or methoxyamines	Plasmatic methoxyamines	CT scan/ RMI	MIBG	Adrenalectomy	Anatomopathology
1	20	None	Elevated	ND	Bilateral	ND	Bilateral	Adenoma, hyperplasia
2	29	Palp, headaches	Elevated	ND	Bilateral	ND	Bilateral	Adenoma
3	20	None	Elevated	ND	Left	Bilateral	Bilateral	Adenoma, hyperplasia
4	22	Palp, hBP	Elevated	Elevated	Right	Right	Right	Adenoma
5	28	Headaches, hBP	Elevated	Elevated	Bilateral	ND	Bilateral	Adenoma
6	29	hBP	Elevated	ND	Bilateral	Bilateral	Bilateral	Adenoma
7	21	Palp, sweating	Elevated	ND	Bilateral	Bilateral	Bilateral	Adenoma, hyperplasia
8	29	None	Elevated	Elevated	Bilateral	Bilateral	Bilateral	Hyperplasia
9	13	Palp, headaches	Elevated	ND	Right	Bilateral	Bilateral	Hyperplasia
10	29	hBP, hypoT	Elevated	ND	Right	Right	Bilateral	Hyperplasia
11	30	hBP	Elevated	ND	Left	Left	Left	Adenoma, hyperplasia
12	18	None	Elevated	ND	Right	Right	Right	Adenoma
13	22	None	Elevated	Elevated	Right	Right	Right	Adenoma
14	12	Sweating, hBP	Elevated	ND	Ectopic	Ectopic	Ectopic	Adenoma

Palp, palpitations; hBP, high blood pressure; hypoT, hypotension; ND, not determined.

were treated with hydro- and fludrocortisone presented Addisonian crisis during the follow-up.

Discussion

In retrospective collaborative studies (7–11), as in our series, asymptomatic pheochromocytoma occurred in about 1/3 of cases, and only a minority of the pheochromocytoma clinically expressed all of the classical symptoms: none in our series and only 15.6% in a large series of 100 pheochromocytomas (7).

As in other studies, the determination of urinary methoxyamines enables the biological diagnosis of pheochromocytoma (18–21). Total urinary catecholamines and/or methoxyamines were always elevated in our series even in asymptomatic patients. Hence, this shows the necessity to use these parameters systematically in order to detect pheochromocytoma in genetically predisposed subjects, i.e. MEN 2A and 2B kindred, but also in patients considered as FMTC only. Such systematic screening would make it possible to avoid deaths due to undiagnosed pheochromocytoma. In the retrospective EuroMen study, 300 MEN 2 pheochromocytomas were analyzed: 25 of 39 deaths were linked to pheochromocytoma (64.1%), ten of which were undiagnosed (9). Casanova and colleagues previously reported similar results in a survey of 100 pheochromocytomas (7). However, the problem with this type of screening is the unreliability of 24 h urine collection. In this context, we do not have sufficient experience of plasma methoxyamine determination, a method which is described as more effective than the urinary analysis we used (22–25). In only four of our 14 patients presenting pheochromocytoma did the plasma analysis results (22) correspond to those of urinary assays (Table 2).

In our series, after the biological diagnosis, the combination of CT scan or MRI and MIBG scintigraphy allowed correct localization of pheochromocytoma in 13 of 14 patients. For two of these patients, MIBG scintigraphy correctly assessed the bilateral adrenal involvement whereas CT scanning revealed only unilateral involvement. Increased MIBG intake was associated with adrenomedullary hyperplasia which was not visualized by CT scan. In a comparative study, Maurea and colleagues showed that computed tomography and RMI are more sensitive (100%) than MIBG scintigraphy (82%) in patients who had no previous surgery; they recommend CT scanning and RMI for the accurate localization of adrenal tumor sites (26). However, even if MEN 2 pheochromocytomas are rarely ectopic or malignant, we believe that MIBG is helpful in detecting precocious hyperplasia and in localizing the rare extra-adrenal pheochromocytoma. Moreover, MIBG must be performed to confirm the functional activity of adrenal masses detected by CT scanning or MRI, because the high frequency of incidentalomas in the general population (27). It will

Figure 1 Relationship between the age of the 87 non-index MEN 2 gene carriers and (i) the moment of the determination of genetic status (white columns), (ii) the diagnosis of medullary thyroid carcinoma (MTC) (grey columns), (iii) the diagnosis of pheochromocytoma (black columns).

also be essential if CT scans or RMI are negative in spite of clinical and biochemical evidence or for patients with previous surgery and/or with recurrence (26). Ultrasound examination is not sensitive enough to detect small pheochromocytoma and thus is not useful in early detection and follow up (7, 10).

In retrospective series, pheochromocytomas are bilateral in 60–80% of cases, and in the case of unilateral involvement contralateral pheochromocytoma may occur more than 20 years later. We also observe a bilaterality in 78.6% of cases despite the young age of our population. Only three pheochromocytomas remain unilateral after a delay of 3, 3.5 and 5 years; therefore, it is expected that a bilateral pheochromocytoma may appear much later in these patients.

Pheochromocytomas require adrenalectomy. Bilateral adrenalectomy must be performed in the case of bilateral pheochromocytoma. For unilateral pheochromocytoma, given the high frequency of relapse on the remaining adrenal, primary bilateral adrenalectomy may be indicated. This choice is difficult and depends essentially on the possibilities of long-term clinical and biological monitoring of the patient, to avoid on the one hand Addisonian crisis and on the other unrecognized contralateral pheochromocytoma.

Current imaging techniques make it possible to localize the pheochromocytoma and determine the unilateral or bilateral involvement (28). Thus it is now possible to treat unilateral and bilateral pheochromocytoma by video-assisted adrenalectomy, and to repeat

Figure 2 Chronology of appearance of medullary thyroid carcinoma (MTC: ●) and pheochromocytoma during the follow up of the 14 patients with pheochromocytoma. Pheochromocytoma of the right adrenal gland is represented as ▼, of the left adrenal gland as ▲, and ectopic pheochromocytoma as □. The follow-up of the patients is represented as a continuous arrow for MEN 2A, and a dashed arrow for MEN 2B.

this approach later on the contralateral adrenal if necessary (29–31). In order to preserve adrenocortical function, certain authors have recently recommended adrenal-sparing surgery (32–37). We do not have experience in this type of surgery, which represents a potential advantage in preventing the adrenal insufficiency resulting from bilateral adrenalectomy (36). In our series, no Addisonian crisis was observed but it is, of course, the major complication that we have to prevent in total bilateral adrenalectomized patients. The possible risks of the adrenal sparing surgery are leaving adrenomedullary cells in place and their spillage during surgery which can induce *in situ* or ectopic recurrences of pheochromocytoma as previously described (32–37). As biological and imaging pheochromocytoma diagnosis are becoming more and more precocious, the number of asymptomatic patients is logically growing. Adrenal-sparing surgery could prove useful in the future to avoid adrenal insufficiency.

The most significant differences between our prospective and retrospective series concern the observed frequency of pheochromocytoma and their chronological relationship to MTC diagnosis.

In our series, the proto-oncogene *RET* mutations are those most frequently found in the literature (6, 10, 13, 38): the 12 MEN 2A patients with pheochromocytoma had a 634 *RET* mutation and the 2 MEN 2B patients a 918 *RET* mutation. With these genotypes, the occurrence of pheochromocytoma in the kindred is about 50%. This corresponds to what we observed for all of the families included in the GETC data base (13) from which the kindred in this study originate. Nevertheless, mutations more recently described and less frequently encountered (39–41), for which the prevalence of pheochromocytoma is not yet established, are not present in our study.

Because of the early MEN 2 genetic diagnosis in this study (14 ± 7 years) made through systematic familial screening, the patients included in our series are younger at the time of pheochromocytoma diagnosis (23.2 ± 6 years) than those of the previous studies (between 32 and 39 years old) (7–10). This may also explain the lower pheochromocytoma frequency of patients presenting a pheochromocytoma, 16% instead of the 50% reported in retrospective studies with this type of mutation (1, 10). Only 8% of our population presented pheochromocytoma at the time of MEN 2 diagnosis versus 34.7–73% in the other series. Moreover, simultaneous diagnosis occurred in the oldest of our population, i.e. the 26–30 year age range (7–10). Taking into account the frequency of pheochromocytoma expected in these genotypes, we foresee that other cases of pheochromocytoma will occur in our population.

All of our patients had MTC, which was biologically detected by basal or pentagastrin calcitonin measurement and proved by anatomopathological findings at

the time of genetic diagnosis of MEN 2. This demonstrates once more the early occurrence of C cell pathology in MEN 2 (42). No pheochromocytoma was diagnosed before MTC in this study, contrary to the findings of the retrospective series which showed that pheochromocytoma could be diagnosed before MTC in 9–25.1% of patients. However, the MTC diagnosis needs a sensitive and specific immunometric assay for calcitonin measurement which had not been systematically used in some of the previous studies (7–10). Moreover, we only considered non-index MEN 2 patients in which all components of the disease were systematically evaluated.

Conclusions

The MEN 2 diagnosis is based on the identification of gene carrier status in the affected kindred. The at-risk subjects bearing the pathological *RET* mutation will thus require surgical treatment of their identified lesions. The MTC is the first lesion which occurs and requires surgical treatment very early in infancy. As soon as the diagnosis of MEN 2 is assessed, annual determination of urinary and/or plasma methoxyamines should be performed to detect pheochromocytoma. Imaging procedures should be carried out when biological tests are positive. CT scan or RMI are essential to localize the adrenal tumors. MIBG confirms the functional nature of adrenal mass and is helpful in detecting hyperplasia and localizing the rare extra-adrenal pheochromocytoma.

This type of monitoring is also necessary after unilateral adrenalectomy to detect foreseeable contralateral pheochromocytoma. Although the presence of *RET* mutations at codon 634 and 918 are reported to be strongly associated with the presence of pheochromocytoma, it is necessary to systematically screen for pheochromocytoma whatever the *RET* mutation identified in the MEN 2 kindred.

Taking into account its potential risks, adrenalectomy is needed as soon as pheochromocytoma is detected.

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