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ORIGINAL ARTICLE

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Primary hereditary medullary thyroid carcinoma – C-cell morphology and correlation with preoperative calcitonin levels

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Abstract Early thyroidectomy offers an opportunity of preventing the development of medullary thyroid carcinoma (MTC) in patients at risk for hereditary MTC. We investigated the thyroid glands of 32 patients with hereditary MTC to identify the changes in C-cell morphology and to correlate these with plasma calcitonin (CT) levels and with clinical data. The entire thyroid gland was processed for histological examination including immunostaining for CT. All glands revealed C-cell hyperplasia (CCH), and MTC was found in 21 patients (66% of 32, youngest patient 6 years, youngest with lymph node metastases [LNM] 17 years). The transition from CCH to MTC was characterized by destruction of the follicular basement membrane and by diminished intensity of CT immunostaining. Normal plasma CT levels after provocation with pentagastrin were found only in patients with CCH. Basally elevated plasma CT levels were restricted to MTC. LNM were only found in multifocal tumours at least 4 mm in diameter. It is not yet clear whether or not CCH in patients at risk for hereditary MTC is a neoplastic change, but in these patients the term 'C-cell hyperplasia' is of doubtful value. All *MEN* gene carriers reveal CCH, and almost all of them will develop multifocal MTC, so that CCH is probably a precursor lesion of an indubitably malignant tumour. Prophylactic thyroidectomy is justified at the age of 6 to anticipate development of a MTC. Lymphadenectomy is necessary in children if they are older than 10 years or have elevated plasma CT levels.

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Introduction

About 40 years ago, medullary thyroid carcinoma (MTC) was identified as a clinicopathological entity [12]. The association of thyroid tumours and phaeochromocytomas was observed as early as 1952 [4], but it was not until the early 1960s that the first reports on familial occurrence of thyroid tumours and their frequent association with phaeochromocytoma were published [3, 24]. It was recognized that the familial forms of thyroid carcinomas were most often of the medullary type and the name 'multiple endocrine neoplasia' (MEN) was proposed for the association [25].

For many years, calcitonin (CT) served as the one tumour marker, and its measurement remained the definitive test for prospective diagnosis of MTC [21]. In 1993, germline mutations in the *RET* proto-oncogene were described as the initial step in the development of the MEN2 syndrome [7, 19]. Since more than 90% of gene carriers develop MTC, a 'prophylactic' thyroidectomy is thought to be the best approach to prevent the development of carcinoma in patients at risk. The possibility of performing a thyroidectomy on *MEN2* gene carriers is considered even before their plasma CT levels became pathologic [2, 8, 27, 28].

We have investigated changes of the C-cell morphology in the thyroid glands of 32 patients who underwent thyroidectomy after mutation in the *RET* proto-oncogene was found. We looked for correlations of these changes with plasma CT levels and with clinical data. For 20 of the 32 patients more than one member of the kindred was investigated.

Materials and methods

Patients were divided into two groups named prophylactic and symptomatic. Patients were prophylactic if the *RET* mutation was diagnosed before awareness of any symptoms, if no pathologic alterations of the thyroid gland or lymph nodes were detectable by ultrasound and if the patient was under 25 years of age. All other patients were classified as symptomatic. Thus, the term 'prophylactic' does not exclude either MTC or lymph node metastases (LNM) in the pathological specimen. There were 22 patients the prophylactic group and 10 in the symptomatic group.

The thyroid gland was divided vertically to separate the left and right lobes, and the two halves were sliced horizontally. After fixation in formalin, the entire specimen was embedded in paraffin. Soft tissue adjacent to lymph nodes was processed separately. Haematoxylin and eosin (H&E) and immunhistochemical staining for CT were assessed in each case. For CT immunostaining, the standard avidin–biotin complex peroxidase method was applied using a commercial polyclonal antibody (Immunotech, Marseilles, France). All available H&E slides were reviewed by two of the authors (R.H. and H.J.H.).

The criteria for C-cell hyperplasia (CCH) according to Wolfe at al. [29] and DeLellis [5] were used. These defined CCH as >6 C-cells per thyroid follicle, and/or >50 intrafollicular CT-positive cells in at least one low-power (×100) field.

The values for preoperative plasma CT levels (basal and after stimulation with pentagastrin) were received from various laboratories, and since the normal and threshold values differed considerably between laboratories, data were subdivided into three main groups with either basally and stimulated normal CT (group A), basally normal but stimulated elevated CT (group B) and basally elevated CT (group C), the normal values of the contributing laboratories being taken as reference levels.

Results

A synopsis of clinical, molecular genetic, and morphological data is shown in Table 1. CCH was recognizable in all thyroid glands by H&E, but was more clearly apparent with CT immunostaining. The staining patterns ranged from mild change with unilateral hyperplasia (2 cases, ages 3 and 9 years) to extensive changes with multifocal and bilateral occurrence of large clusters of C-cells. In mild CCH we observed an increased number of C-cells per follicle (focal CCH) and also ring-like C-cell clusters (diffuse CCH) with eccentric or circular intrafollicular proliferation (Fig. 1a, b). In more advanced stages a nodular pattern of hyperplasia was observed, with complete replacement of the pre-existing follicular epithelium. Some cells showed slight nuclear atypia, but the basement membrane

Table 1 Synopsis of clinical, molecular genetic and morphological data [*No*. number, *CT* calcitonin i increased, *CCH* C-cell hyperplasia, *MTC* medullary thyroid carcinoma, *LNM* lymph node metastasis, *Phaeo*. Phaeocromocytoma, *m*. male, *f*. female, *pro*. prophylactic (definition see text), hyperplasia *symp.* symptomatic (definition see text), *n* normal, *i* increased, *n.d*. not determined, + unilateral, ++bilateral, (+/++) mild hyperplasia with focal CCH, *n.i.* not investigated, (*hyp*.) signs of mild hyperplasia]

No.	Age (years)	Sex	Kindred	Indication	Mutation codon (DNA sequence)	CT level basal/stimulated	CCH	MTC	Maximum Size (mm)	LNM[a]	Parathyroid gland	Phaeo.
1	3	m	A1	pro	620 (CGC)	n/i	(+)	–		–	n	–
2	5	m		pro	634 (CGC)	n/i	++	–		–	n.i.	–
3	6	f	B1	pro	618 (GGC)	n/i	(++)	–		–	n	–
4	6	m	A2	pro	620 (CGC)	n/i	(++)	–		–	n	–
5	6	m	E1	pro	634 (CGC)	i/i	++	+	2	–	n.i.	–
6	7	f	C1	pro	634 (TAC)	i/i	++	++	2,5	–	n	–
7	7	f	E2	pro	634 (CGC)	i/i	++	+	3	–	n.i.	–
8	8	m	B2	pro	618 (GGC)	n/i	++	–		–	n	–
9	8	f		pro	611 (TAC)	n/n	(++)	–		–	n.i.	–
10	9	f	A3	pro	620 (CGC)	n/i	(+)	–		–	n	–
11	10	m	G1	pro	634 (TTC)	n/n	++	–		–	n	–
12	11	m	C2	pro	634 (TAC)	i/i	++	++	5	–	n	–
13	11	f	G2	pro	634 (TTC)	n/n.d.	++	+	1,2	–	(hyp.)	–
14	13	f		pro	634 (CGC)	n/i	++	++	5	–	n	–
15	13	m	F1	pro	620 (AGC)	n/n	(++)	–		–	n	–
16	13	m		pro	634 (TCC)	n/i	++	–		–	n	–
21	13	f	B3	pro	618 (GGC)	n/i	++	++	3,5	–	n.i.	–
17	15	m	B4	pro	618 (GGC)	n/i	++	+	3	–	n	–
18	18	m	F2	pro	620 (AGC)	n/i	++	–		–	n	–
19	20	m		pro	634 (CGC)	i/i	(++)	++	4	+(2/74)	(hyp.)	–
20	20	f	D1	pro	634 (TAC)	n/i	++	++	8	+(1/83)	n	++
22	23	f		pro	634 (TTC)	i/	++	++	7	–	(hyp.)	–
23	17	f	D2	symp.	634 (TAC)	i/i	++	++	40	+(2/56)	n.i.	++
24	24	f		symp.	634 (TAC)	i/i	++	++	10	–	n.i.	–
25	27	m		symp.	634 (TAC)	n/i	++	++	7	+(1/130)	n	–
26	31	f	A4	symp.	620 (CGC)	n/i	++	++	5	–	n.i.	–
27	34	f		symp.	634 (TTC)	i/i	++	++	7	–	n.i.	–
28	34	m	D3	symp.	634 (TAC)	i/i	++	++	5	+(2/98)	n.i.	++
29	41	m	D4	symp.	634 (TAC)	i/i	++	++	8	+(10/98)	n	–
30	47	f		symp.	634 (TAC)	i/	++	++	22	–	n.i.	–
31	51	m		symp.	634 (CGC)	i/	++	++	7	+(2/7)	n	++
32	53	f		symp.	768 (GAC)	i/i	++	++	6	–	n.i.	–

[a] Figures in round brackets show no. of lymph node metastases/no. of lymph nodes investigated

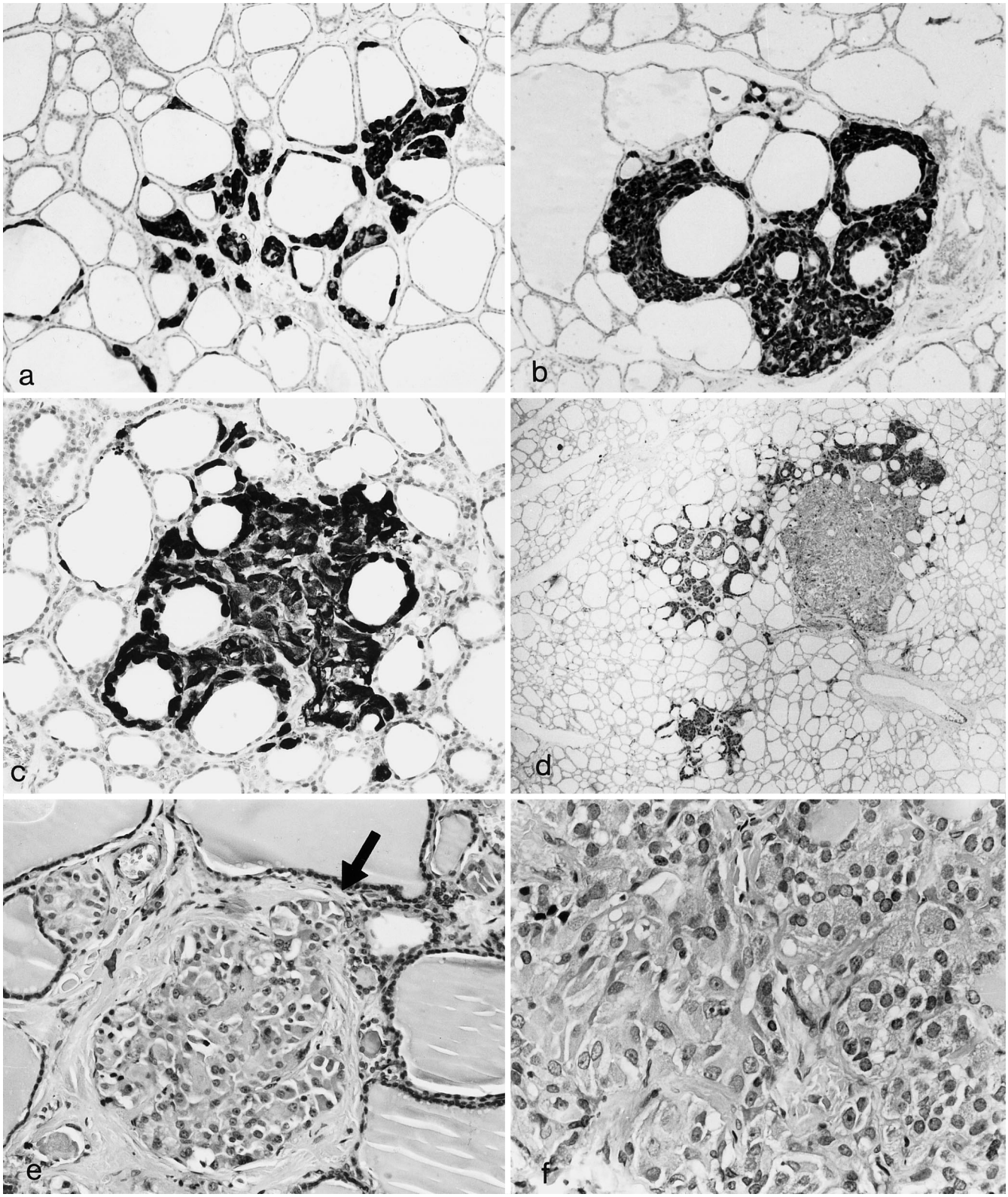

Fig. 1 **a** Mild form of CCH with focal replacement of the pre-existing follicular epithelium by proliferating C-cells. CT immunostaining, original magnification ×100 **b** More advanced stage of CCH with half-moon and ring-like C-cell clusters. Their smooth outlines suggest the follicular basal lamina to be intact. CT immunostaining, original magnification ×100 **c** Both focal CCH and diffuse spreading of C-cells between the pre-existing follicles indicates the transition to an invasive tumour. CT immunostaining, original magnification ×200 **d** Small MTC (middle right) with diminished CT staining intensity accompanied by extensive CCH in the vicinity of the tumour. CT immunostaining, original magnification ×25 **e** Small focus of MTC with moderate nuclear atypia of C-cells, remarkable desmoplasia surrounding the tumour focus and infiltrative growth pattern (*arrow*). HE, original magnification ×400 **f** Increasing cellular atypia within a small MTC focus with enlarged polygonal or fusiform tumour cells, showing variations in nuclear form and size as well as prominent nucleoli. HE, original magnification ×400

Table 2 Correlation between age, size and number of thyroid gland tumours for all patients with medullary thyroid carcinoma (n=21).

Age (years)	Size (mm)		Number of tumours per patient	
	Median	Mean (range)	Median	Mean (range)
1–9 (*n*=3)	2.5	2.5 (2–3)	1.5	1.6 (1–2)
10–19 (*n* =6)	4.25	9.6 (1.2–40)	1.5	1.6 (1–2)
20–29 (*n* =5)	7	7.2 (3.5–10)	3	3.2 (2–7)
>30 (*n* =7)	7	8.6 (5–22)	2	2.7 (2–5)
Correlation between size and number of tumours in patients without and with LNM:				
Without lymph node metastasis (*n* =14)		6.0 (2–22)		2.1 (1–7)
With lymph node metastasis (*n* =7)		11.3 (4–40)		3.0 (2–7)

Table 3 Correlation between preoperative calcitonin levels and morphology of C-cells (n=31)[a]

Group	Calcitonin		No.	Indication for operation	Morphology		
	basal	Stimulated			CCH	MTC pN0	MTC pN1
A	Normal	normal	3	pro.	*n*=3		
B	Normal	elevated	12	pro.	*n*=8	*n*=3	*n*=1
			2	symp.		*n*=1	*n*=1
C	Elevated	Elevated	6	pro.		*n*=5	*n*=1
			8	symp.		*n*=4	*n*=4

[a] One patient was excluded because of lack of information on stimulated plasma calcitonin level (basal calcitonin level within no rmal range)

surrounding the follicle was intact. In large nodular clusters of C-cells, obvious disruption of the follicular basement membrane marks the transition to early invasive MTC (Fig. 1c, e). This step is reflected by a loss of follicular architecture and an increased amount of connective tissue surrounding the C-cells. The individual C-cells did not show consistent cellular changes accompanying the step from CCH to small foci of MTC. Advanced-stage hereditary tumours closely resembled sporadic MTC.

In our trial, the youngest patient with MTC was aged 6 years. Multifocal MTC was present in more than 50% of patients aged 10–20 years and in almost 100% of patients older than 20 years. The youngest patient with LNM was 17 years old. Only bilateral tumours at least 4 mm in diameter developed LNM (Table 2). About 50% of patients older than 20 years of age had LNM. The youngest patient with bilateral phaeochromocytoma was aged 17 years. The size of the primary tumour and the number of tumour foci per patient increased with age (Table 2). The mean size and number of tumour foci was lower in patients without LNM than in patients with LNM (not significant). Immunostaining for CT showed pronounced decoration of hyperplastic C-cells. In early MTC a clear dimininution of the staining intensity was observed in unequivocally malignant tumours compared with CCH foci in the vicinity of the tumour (Fig. 1d). In addition, there was definite variation within individual tumours and between different growth patterns when different MTCs were compared. The most marked reduction was found in stroma-rich tumours.

Stimulated normal CT was found in 3 out of 22 cases classified as prophylactic, which revealed mild forms of CCH. Basal elevation of CT was observed exclusively in patients with MTC and in all cases with metastases. Stimulated abnormal CT levels were demonstrated in 8 patients with CCH (prophylactic group) and in 20 with MTC (prophylactic and symptomatic groups; Table 3).

Discussion

The data on the numbers of patients with MEN2 syndrome-associated *RET* proto-oncogene mutation who develop MTC in the course of their lives vary considerably. Some authors assume that 95% or more of *MEN2* gene carriers will develop MTC [9], and our study supports this assumption by showing MTC in more than 50% of patients aged 10–20 years and in 100% of patients older than 20 years. In the literature, the earliest occurrence of hereditary MTC (excluding MEN2B) was reported in a child aged 2.8 years [26] and the earliest metastasis at the age of almost 6 years [10]. Our youngest patient harbouring MTC was 6 years old at the time of operation, and the youngest patient with LNM was 17 years old.

Mild forms of CCH are reported to be difficult to distinguish from upper limits of normal C-cell density [6]. Recently it has been shown that both healthy infants and adults may present CCH according to the established criteria for adults [11]. Using morphological criteria alone we cannot prove that the mild unilateral CCH found in 2 of the children in our study is caused by the initial RET mutation and indicates a pathologic change. The elevated CT level after stimulation would be a point in favour of this interpretation, but a false-positive response to penta-

gastrin stimulation has been reported in *RET*-mutation-negative members of MEN 2a families [17]. These data emphasize the necessity of mutational analysis in patients with mild forms of CCH, to rule out a hereditary form of MTC. Assuming that there is a step by step process from CCH to MTC in hereditay MTC with a low rate of progression, at least in a few cases, the finding of very mild forms (unilateral) of CCH in young children is neither unusual nor unexpected.

The transition from hyperplastic CCH to an invasive tumour is defined by the destruction of the follicular basement membrane [5, 6, 15, 20]. In practice, it is difficult and sometimes impossible to differentiate clearly between a nodular hyperplasia and a nodular infiltration pattern in small MTC [20]. Other features, such as desmoplasia, cytological atypia, and a diminished staining intensity with antibodies against CT, are helpful but equivocal signs. When present in association with MTC, the hyperplastic C-cells were mostly cytologically identical to the invasive component. In the past, various markers have been used to differentiate between CCH and MTC, including selected monoclonal antibodies against CEA and histaminase [22]. Additional markers have also been used to highlight differences between physiological CCH accompanying other tumours and inflammatory diseases of the thyroid gland [14, 20]. However, the latter are not useful for MEN-affected patients, and the former are not demonstrably reliable.

After gross examination, we suggest histological processing of the entire thyroid gland to avoid missing any CCH clusters and small MTC foci. Serial sections and selective stains highlighting basement membrane components, such as silver stains and immunostaining for collagen type IV [18], can be helpful in diagnosis in selected cases.

Despite the clear morphological definition of an early invasive MTC, its biological importance is still obscure. We found clearly malignant tumours in children in the first decade of life, but in most cases the development of metastasis probably takes 20 years or more. However, metastasis has a greater influence on individual prognosis. It is not yet clear whether patients with very small MTC have worse outcomes than patients with multifocal CCH. Probably 100% of the patients with constitutional mutations of the *RET* proto-oncogene develop multifocal CCH, followed by the development of MTC within the CCH in most cases. Does this mean that hyperplasia of C-cells reflects a neoplastic and multifocal but monoclonal change initiated by a germline mutation of the *RET* gene? From this point of view, the terms ‘carcinoma in situ’ or ‘neoplastic CCH’ should be preferred for noninvasive C-cell proliferation in patients at risk of MEN [5, 20]. Genetically determined steps accompanying the transition from CCH to MTC and from nonmetastatic MTC to tumours with metastasis are not defined. These are questions for future studies [13].

Our results show that plasma CT is a reliable marker for the prediction of the extent of C-cell changes. Increased unstimulated plasma CT levels were exclusively linked to carcinomas. Stimulated normal plasma CT levels were found only in patients with mild forms of CCH. In the literature, however, cases of MTC have been described with unremarkable CT levels in patients with MTC and elevated CT levels in patients with neither MTC nor *RET* proto-oncogene mutations [8, 15–17, 28]. The majority of ‘prophylactic’ children showed normal unstimulated and elevated CT levels after provocation with pentagastrin regardless of the histology (pure CCH, MTC without LNM, MTC with LNM). This situation emphasizes the limited value of this test in predicting the clinical course of *MEN2* gene carriers.

The right moment for a prophylactic thyroidectomy is still a matter of discussion. An operation should anticipate the development of a MTC and prevent metastasis. Thyroidectomy before a child starts school (at 5–6 years) is probably best, and extended lymphadenectomy should be included in children who are older than age 10 years or have an elevated CT level. Operating on gene carriers is justified even when they have normal CT levels after provocation [8, 28].

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