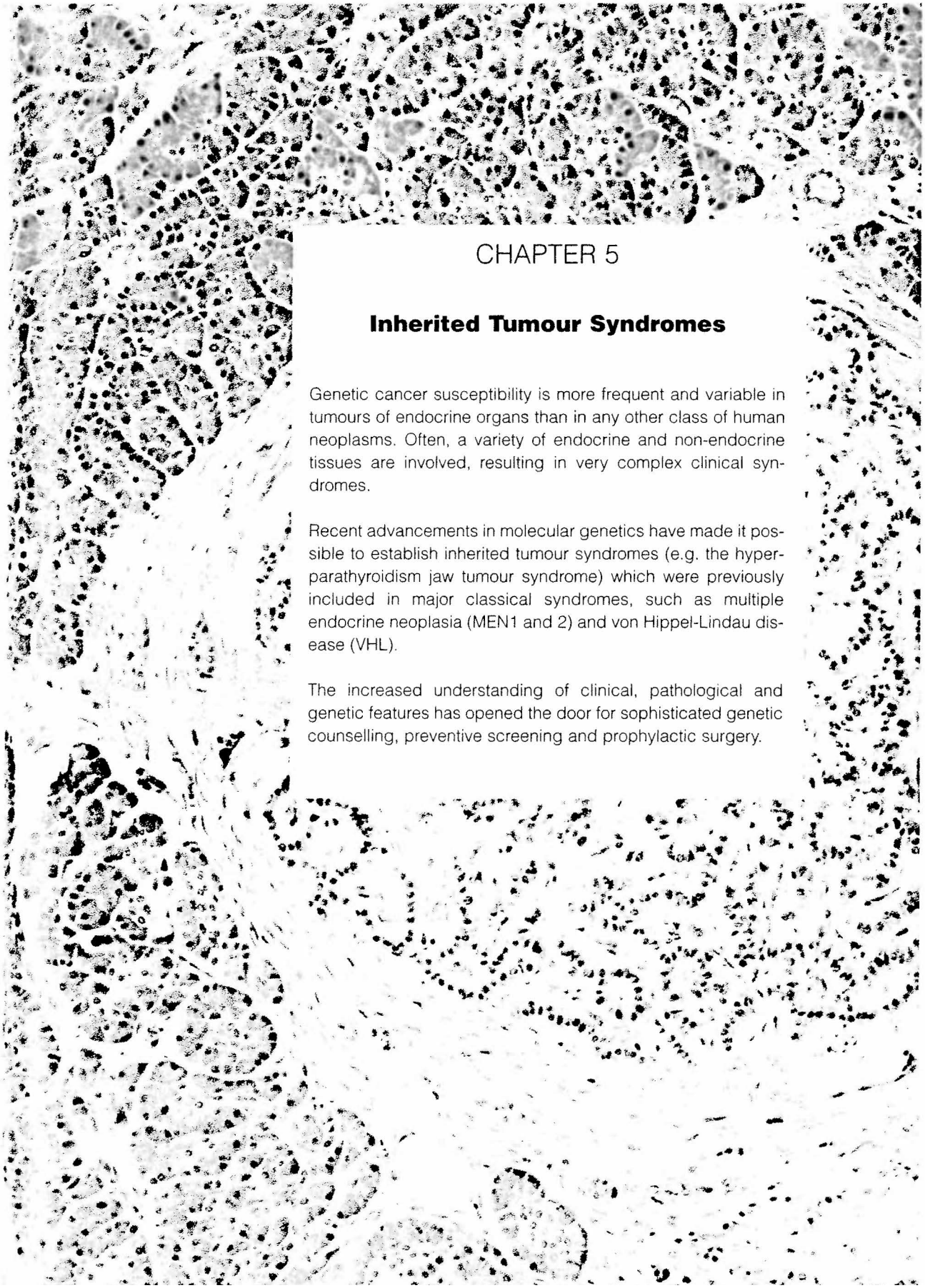

CHAPTER 5

Inherited Tumour Syndromes

Genetic cancer susceptibility is more frequent and variable in tumours of endocrine organs than in any other class of human neoplasms. Often, a variety of endocrine and non-endocrine tissues are involved, resulting in very complex clinical syndromes.

Recent advancements in molecular genetics have made it possible to establish inherited tumour syndromes (e.g. the hyperparathyroidism jaw tumour syndrome) which were previously included in major classical syndromes, such as multiple endocrine neoplasia (MEN1 and 2) and von Hippel-Lindau disease (VHL).

The increased understanding of clinical, pathological and genetic features has opened the door for sophisticated genetic counselling, preventive screening and prophylactic surgery.

Roxane Labs., Inc.
Exhibit 1012
Page 040

Introduction

C. Eng

This chapter discusses the clinical, genetic and pathologic details of endocrine tumours which are components of inherited neoplasia syndromes. In addition to the classical syndromes, such as the two multiple endocrine neoplasia syndromes (MEN) and von Hippel-Lindau disease (VHL), this chapter also contains such entities, which in the past, had been part of sections dedicated to the more "traditional" syndromes. For example, there is a separate section on hyperparathyroidism jaw tumour syndrome which would previously have been discussed with MEN 1. However, with the recent isolation and partial characterisation of the susceptiblity gene, *HRPT2*, this syndrome has come into its own. Similarly, non-MEN 2, non-VHL heritable phaeochromocytoma and paraganglioma would have been briefly discussed in the sections on MEN 2 and VHL. Given the discovery and characterisation of the autosomal genes encoding three of the four subunits of mitochondrial succinate dehydrogenase as susceptibility genes for phaeochromocytomas and paragangliomas, this syndrome was felt to deserve a distinct section as well. Because this is a chapter in a book dedicated to endocrine tumours, a conscious decision was made not to assign Cowden syndrome its own section, but instead to discuss it with all familial nonmedullary thyroid carcinoma predisposition. Finally, there are two particularly notable features in this chapter. Each section discussing a particular type or group of inherited syndrome(s) ends with a subsection on genetic counselling and preventative measures written by a cancer genetic counselor or a practising physician-clinical cancer geneticist. There is also a reference table (below), which gives genetic differential diagnoses based on organ-specific endocrine neoplasias.

Table 5.01
Genetic differential diagnoses by endocrine organ system.

Organ	Histologic Type	Syndromes	Gene
Adrenal	Adrenocortical neoplasia	Li-Fraumeni syndrome	*TP53*
		Carney Complex	*PRKAR1A*
		Beckwith-Wiedemann syndrome	*CDKN1C / NSD1*
		MEN1	*MEN1*
	Phaeochromocytoma	Von Hippel-Lindau disease	*VHL*
		Pheochromocytoma-paraganglioma syndrome	*SDHD, SDHC, SDHB*
		MEN2	*RET*
		Neurofibromatosis type 1	*NF1*
Pancreas	Islet cell neoplasias	MEN1	*MEN1*
		Von Hippel-Lindau disease	*VHL*
Paraganglia	Paraganglioma	Phaeochromocytoma-paraganglioma syndrome	*SDHD, SDHC, SDHB*
		Von Hippel-Lindau syndrome	*VHL*
		MEN2	*RET*
		Neurofibromatosis type 1	*NF1*
Parathyroid	Adenoma / Hyperplasia	MEN1	*MEN1*
		MEN2	*RET*
	Carcinoma	Hyperparathyroidism-jaw tumour syndrome	*HRPT2*
Pituitary	Adenoma	MEN1	*MEN1*
		Carney complex	*PRKAR1A*
Thyroid	Papillary carcinoma ^	Familial adenomatous polyposis syndrome	*APC*
		Cowden syndrome*	*PTEN*
		Carney complex ?	*PRKAR1A*
		Familial site specific non-medullary thyroid cancer syndromes	None yet
	Follicular carcinoma	Cowden syndrome*	*PTEN*
		Werner syndrome	*WRN*
	Medullary carcinoma	MEN2	*RET*

MEN1/2, multiple endocrine neoplasia syndromes.
* The great majority of epithelial thyroid carcinomas seen in Cowden syndrome are folllicular thyroid carcinomas. Occasionally, papillary thyroid carcinomas (PTC) are seen in this syndrome. There is anecdotal evidence that what is commonly referred to as 'PTC' in familial adenomatous polyposis syndrome (FAP) is not identical to classic PTC. However, reclassification awaits systematic studies. For patient care, and hence purposes of differential diagnosis, FAP is still listed under the differential diagnosis of PTC.

Roxane Labs., Inc.
Exhibit 1012
Page 041

Multiple endocrine neoplasia type 2

O. Gimm
C.D. Morrison
S. Suster
P. Komminoth
L. Mulligan
K.M. Sweet

Definition

The multiple endocrine neoplasia type 2 (MEN 2) syndrome is an inherited tumour syndrome with an autosomal dominant pattern of inheritance, caused by germline mutations of the *RET* gene. It is characterised by the coexistence of various endocrine tumours involving the thyroid, the adrenal, and the parathyroids [2121]. In addition, abnormalities affecting various non-endocrine organs/tissues (e.g. intestine, mucosa, cornea, skeleton) may be present. MEN 2 has been clinically subdivided into 3 groups: familial medullary thyroid carcinoma (FMTC), MEN 2A and MEN 2B.

MIM Number

The Mendelian Inheritance in Man (MIM) number for FMTC is 155240, the MIM number for MEN 2A is 171400, and the MIM number for MEN 2B is 162300.

Synonyms

MEN 2 has also been named multiple endocrine adenomatosis (MEA) type 2 or previously abbreviated MEN II, which should no longer be used. MEN 2A is also known as Sipple syndrome [2064]. MEN 2B was also termed MEN 3 [1092]; even less common is the term Wagenmann-Froboese syndrome [665, 2338].

Incidence / Prevalence

The incidence of MEN 2 is unknown. The hereditary form of medullary thyroid carcinoma (MTC) has been assumed to account for about 25% of all MTCs. MTC is believed to account for 5-10% of all thyroid malignancies. The incidence of thyroid cancer has been assumed to be 1-3/100,000 per year. Hence, MEN 2 may have an incidence of approximately 1.25–7.5/10,000,000 per year. Its prevalence is believed to be about 1/35,000. The female to male ratio in MEN 2 is roughly 1:1, in some studies a slight predominance of the female gender has been reported.

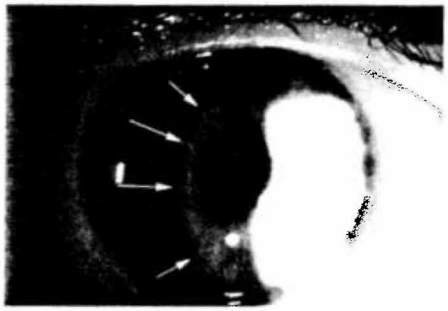

Fig. 5.02 Corneal nerves in a patient with MEN2B syndrome.

Diagnostic criteria

Besides MTC, patients with MEN 2A may develop phaeochromocytoma and/or primary hyperparathyroidism. Patients with MEN 2B may develop phaeochromocytoma, neuromas of the tongue and/or ganglioneuromatosis of the intestine, a marfanoid habitus and/or medullated corneal nerve fibres. None of these latter phenotypes need be present in MEN 2B nor are they pathognomonic [742,746, 1778], i.e. they have been also been reported in patients without MTC or MEN 2B-specific *RET* mutations although it is difficult to determine if such clinical features are "over-called" once a clinical suspicion of MEN 2 is raised. Clinically evident primary hyperparathyroidism is not part of MEN 2B. By definition, patients with FMTC develop MTC only.

Since the identification of RET as the MEN 2 susceptibility gene in 1993, the definitive diagnosis of MEN 2 relies almost exclusively on germline RET mutation analysis. Of note, RET mutation analysis has to be performed in any patient with MTC and is also recommended in patients with phaeochromocytoma [1585], irrespective of age of the patient, the absence of accompanying disease features or family history in order to identify index patients (probands) and to enable at-risk relatives timely diagnosis and therapy.

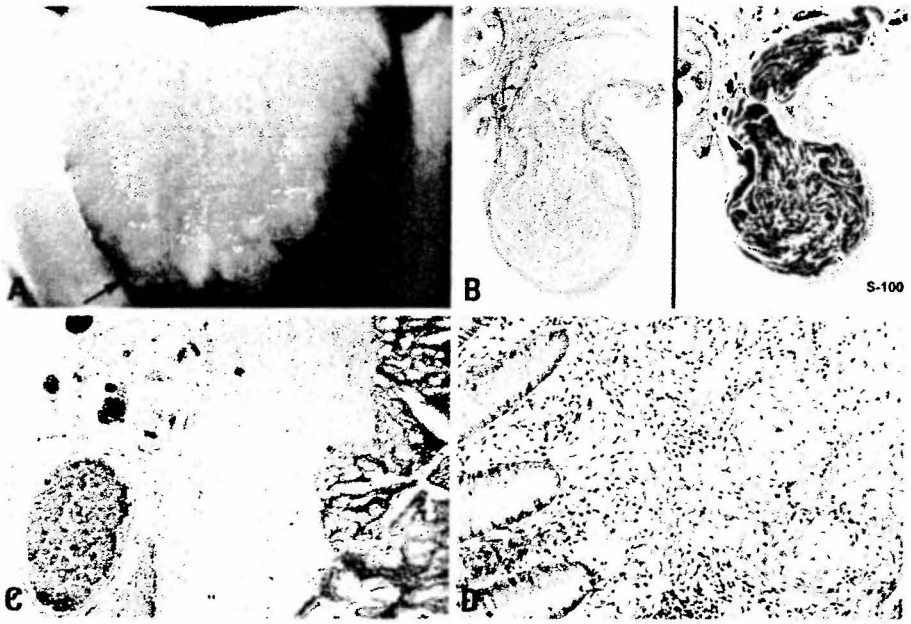

Fig. 5.01 Multiple endocrine neoplasia type 2b. **A** Ganglioneuromatosis of the tongue in a MEN2B patient (arrows). **B** Histology of ganglioneuroma of the tongue. **C** S100 Immunostaining of ganglioneuromatosis of the gallbladder. **D** Microscopic aspect of a ganglioneuromatosis in the appendix of a MEN2B patient.

Roxane Labs., Inc.
Exhibit 1012
Page 042

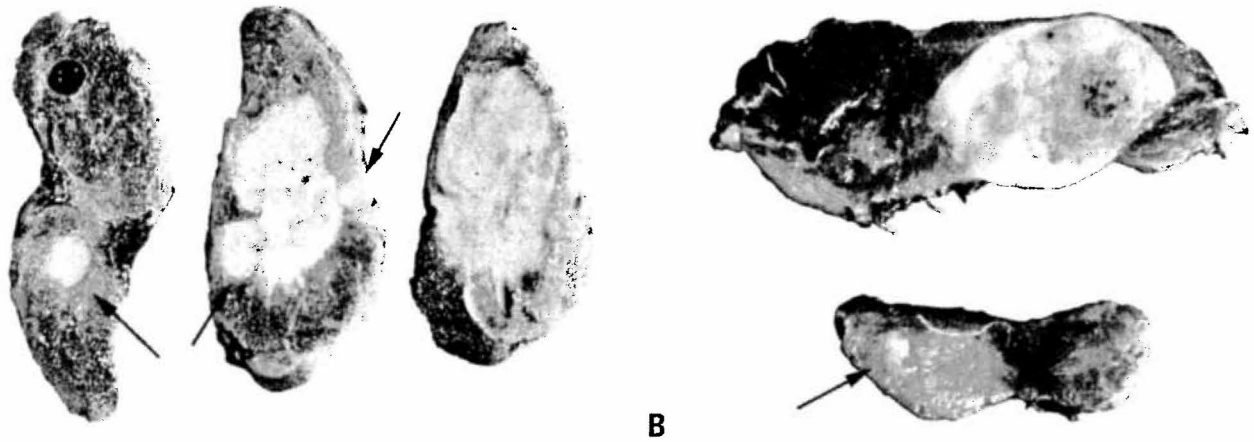

Fig. 5.03 Multiple endocrine neoplasia type 2 (MEN 2). **A** Macroscopic aspect of a multifocal (arrows) familial medullary thyroid carcinoma. **B** Bilateral medullary thyroid carcinoma.

Medullary thyroid carcinoma

Age distribution / penetrance

About 70% of patients with MEN 2A develop clinically apparent MTC by the age of 70 years {1762}. By the age of 35 years, the biochemical penetrance reaches 100%. Of note, MTC has been found in young children age 10 years and below {526,1309,2373}, in MEN 2B even at the age of 6 months {2210}. Patients with FMTC diagnosed with thyroid tumours are on average older than patients with MEN 2A.

Clinical features

Thyroid nodules may be the first clinical sign of MTC. Routine preoperative calcitonin measurement in any patients having a thyroid nodule may identify MTC preoperatively {1670,1828,2318}. Medullary carcinoma of the thyroid metastasizes early to lymph nodes and distant organs (mainly liver, lung and bone). At primary operation, generally more than 50% of index patients already have lymph node metastases. Hence, metastases may be the initial symptom of patients with MTC. In the case of high serum calcitonin levels, symptoms may arise from diarrhoea not responding well to common anti-diarrhoeic drugs. It is relatively rare to present with phaeochromocytoma or hyperparathyroidism.

Pathology

The histopathologic features of MTC in MEN 2 are virtually indistinguishable from those observed in the sporadic cases. Certain features, however, differ in the two ways. As noted earlier, MTC in MEN 2 tends to occur at a younger age and is typically bilateral and multicentric {190,200}. The tumours are typically well circumscribed but unencapsulated, with a tan-pink, soft to rubbery cut surface. The smaller lesions tend to arise at the junction of the upper and middle third of the thyroid lobes, corresponding to the areas containing the highest concentration of C cells. Larger lesions can occupy the entire lobe and infiltrate the perithyroidal tissue.

Another feature that distinguishes the sporadic form of MTC from that in MEN 2 is the frequent presence of *C-cell hyperplasia* in the latter {200,1728,2407}. In MEN 2 patients, foci of C-cell hyperplasia are typically present in the vicinity of the tumours as well as in areas away from the main tumour mass. The finding of C-cell hyperplasia may thus serve as a morphologic marker for MEN 2-associated MTC. The definition of C-cell hyperplasia remains controversial. Morphologically, the process can be nodular or diffuse {480,2406}. In diffuse hyperplasia, C-cells are increased and diffusely scattered throughout the thyroid parenchyma. In nodular hyperplasia, C-cells occur in clusters that may obliterate the follicular spaces. Most authors require the presence of clusters of more than 6 cells in several foci from both lobes to make the diagnosis of nodular C-cell hyperplasia {1470,2408}. In the diffuse form, an increase of over 50 C-cells per low-power field in both thyroid lobes is the most commonly accepted criterion {1862}. Use of special stains, including calcitonin and chromogranin immunostains, may be of aid for highlighting these cells in cases of C-cell hyperplasia. However, at present, genetic testing is the most accurate method for identifying familial and MEN 2-associated C-cell lesions, and is more reliable than morphologic identification of C-cell hyperplasia {1309,1734,2373}. C-cell hyperplasia has also been described in association with non-medullary thyroid carcinoma, follicular adenoma, lymphocytic thyroiditis and solid cell nests {30,345,797}. Such cases have been regarded as a reactive physiologic process unassociated with malignant potential {1728}.

The histologic appearance of MTC in MEN 2 mirrors that of the sporadic cases. A wide range of cytologic features and histologic growth patterns characterize the tumours. As with the sporadic cases, a solid or compartmentalized ("organoid") growth pattern predominates. The tumour cells may be round, oval, polygonal or spindled, and generally contain abundant granular eosinophilic cytoplasm. The nuclei are uniform, round to oval, with occasional pleomorphism and multinucleation. Mitotic figures are usually scarce, particularly in the smaller tumours. Stromal deposits of amyloid and calcium deposits resembling psammoma bodies are also frequent findings. Unusual morphologic variants that can mimic other tumours have also been described.

The histologic diagnosis of MTC can be confirmed with the use of immunohistochemical stains. Calcitonin and calcitonin gene-related peptide (CGRP) represent the most sensitive markers for these tumours, although they are not

Roxane Labs., Inc.
Exhibit 1012
Page 043

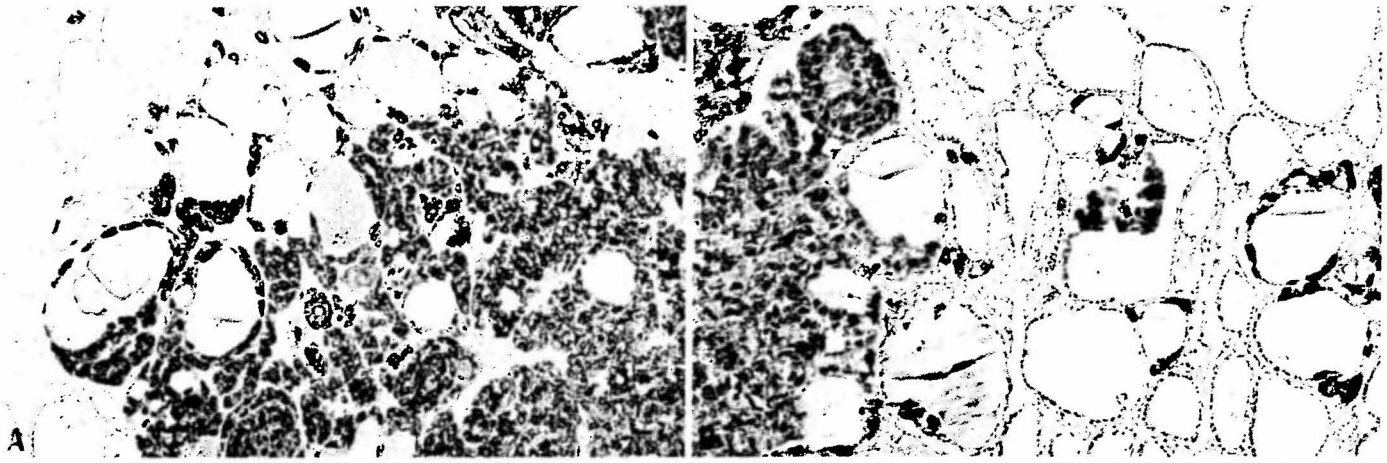

Fig. 5.04 Multiple endocrine neoplasia type 2 (MEN 2). **A** Medullary thyroid carcinoma in a MEN2 patient. Calcitonin immunohistochemistry. **B** C-cell hyperplasia adjacent to a medullary thyroid carcinoma of a patient with MEN2 (calcitonin immunostaining).

entirely specific and can be expressed in other conditions [481,2051,2477]. Calcitonin positivity can be focal and restricted to only a few tumour cells. Also, a small subset of MTC (approximately 1.5%) may be negative for calcitonin [909].

Two additional markers of value for the diagnosis of MTC are chromogranin and CEA; strong expression of the latter has been identified in some studies as associated with a worse prognosis [1981]. A variety of other markers, including low-molecular weight cytokeratin, vimentin, neuron-specific enolase, synaptophysin, somatostatin, and numerous other peptides have been identified in these tumours but are not specific for MTC and are therefore of limited diagnostic value [1862].

Prognosis and prognostic factors

Patients with hereditary MTC overall appear to have a better prognosis than patients with sporadic MTC. However, this may be due to the younger age at diagnosis because of surveillance, i.e. lead time bias. Patients with MEN 2B are believed to develop the most aggressive form of MTC; however, a recent study did not confirm this assumption [1259]. The 5-year survival rate in MTC is about 80-90%, the 10-year survival rate is about 60-70% [177,877]. Prognostic factors may be tumour stage [177,525], and postoperative calcitonin level [525,724]. The studies that tie the presence of somatic M918T mutation and prognosis [817,1347] are deeply flawed as somatic mutation analyses were performed on either primary MTC or metastases.

Phaeochromocytoma

Clinical features

The most common symptoms are hypertension, headache, tachycardia, and sweating. Patients with phaeochromocytoma may present with orthostatic dysregulation. In patients with MEN 2, phaeochromocytoma rarely (10%) precedes the development of MTC [324] and, hence, is either diagnosed synchronously or metachronously during follow-up. Due to the genetic origin of the disease, both adrenal glands may be affected.

Biochemically, phaeochromocytoma can be diagnosed by measuring elevated levels of free catecholamines (epinephrine, norepinephrine) in 24-hour urine. Alternatively, their metabolites (e.g. vanillylmandelic acid) may be measured. Even more sensitive (>95%) seems to be the determination of plasma metanephrines or chromogranin A [550]. Once diagnosed biochemically, the localization and the extent of the phaeochromocytoma needs to be determined. Enlarged adrenal glands are almost always present if the patient is symptomatic. In these cases, computed tomography and/or magnetic resonance imaging may be helpful in determining both localization and extent of the phaeochromocytoma. While both imaging techniques have a high sensitivity (reaching up to 100%), their specificity is rather low (70%). Also, they may fail to identify extra-adrenal phaeochromocytoma. However, extraadrenal phaeochromocytomas are a relatively unusual event in MEN 2. In these instances, ^{131}I-metaiodobenzylguanidine (MIBG) may be very helpful. While its sensitivity is only about 80%, its specificity reaches nearly 100%.

Pathology

The majority of patients with MEN 2A and MEN 2B exhibit bilateral diffuse or nodular adrenal medullary hyperplasia as a precursor of phaeochromocytoma. Adrenomedullary involvement is not a feature of true FMTC. Diffuse medullary hyperplasia is defined as the expansion of the medulla into the alae or tail of the gland with or without nodule formation, enlargement of the adrenal medulla beyond the normal ratio of cortical area to medullary area of 4:1 and a two- to three-fold increase in medullary volume and weight as compared to age- and sex-matched controls [483,1881]. Nodules larger than 1 cm in diameter are considered phaeochromocytomas, while smaller nodules are defined as nodular medullary hyperplasia [316]. However, these criteria are arbitrary and remain to be confirmed by molecular analyses. Macroscopically, nodules of adrenomedullary hyperplasia are typically grey to tan and soft in texture. On microscopic examination there is often a mixed pattern of diffuse and nodular hyperplasia expanding into the tail of the gland and there may be intermingling of medullary and adrenocortical cells. Nodules vary in size, usually lack true encapsulation, may show a "nodule in nodule" appearance and outlines of neighbouring nodules may be partially molded [1192]. Cellular, architectural and immunohistochemical features of hyperplastic lesions are similar to those

Roxane Labs., Inc.
Exhibit 1012
Page 044

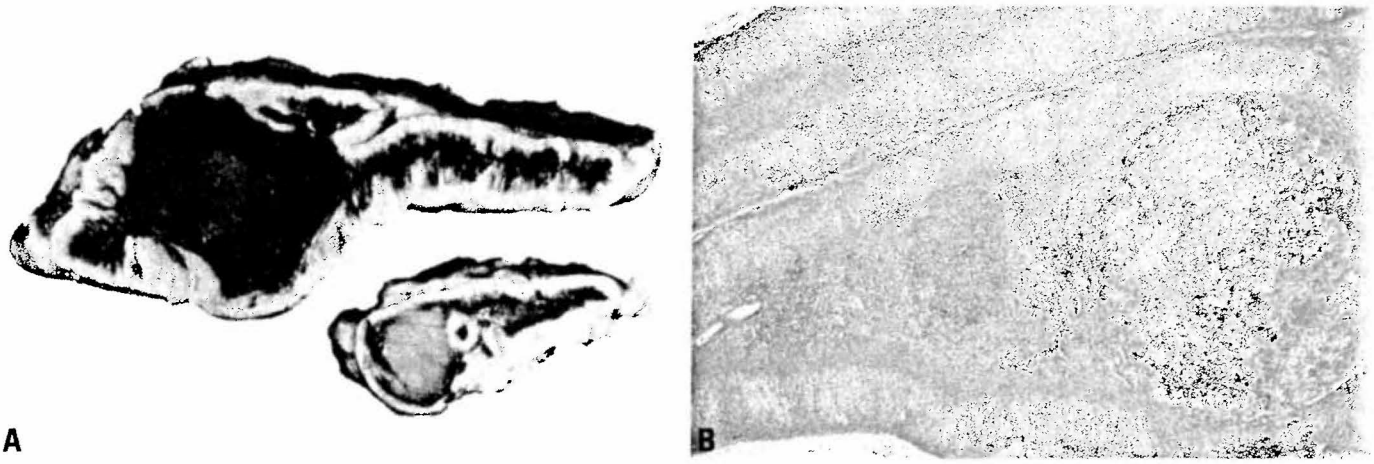

Fig. 5.05 Multiple endocrine neoplasia type 2 (MEN 2). **A** Macroscopic view of multifocal phaeochromocytoma and adjacent nodular adrenomedullary hyperplasia in a patient with MEN2. **B** Close up of nodular adrenomedullary hyperplasia (nodules < 1 cm) adjacent to phaeochromocytoma in a patient with MEN2.

seen in phaeochromocytomas.

Phaeochromocytomas in MEN 2 patients are mostly multicentric and bilateral, vary in size and are confined to the adrenal medulla {2363}. However, some extraadrenal tumours and a phaeochromocytoma arising from an accessory adrenal gland have also been described {1048,1610}. Smaller tumours are often accompanied by diffuse and nodular adrenomedullary hyperplasia but larger tumours, as sporadic phaeochromocytomas, show expansive growth with compression of the adrenal cortex and overgrowth of the hyperplastic medulla. Histologically, MEN 2-associated phaeochromocytomas are similar to sporadically occurring counterpart tumours. However, some studies reported a higher frequency of insular pattern, large or pleomorphic cells with vacuolated or granular cytoplasm, prominent nucleoli and hyaline globules in MEN 2A tumours than in sporadic phaeochromocytomas {751,1121}. Occasionally, lipid degeneration may histologically mimic an adrenal cortical tumour {2282}. Melanin pigmentation, the presence of phaeochromoblasts as well as calcospherites have also been described {1221}.

Immunohistochemically, MEN 2-associated phaeochromocytomas exhibit a similar expression pattern of neuroendocrine and other markers as sporadic tumours {1132}. However, some have reported differences including higher levels of corticotrophin hormone, lower levels of VIP and more S100 protein-positive cells in familial tumours when compared to sporadic phaeochromocytomas {1329,1534}. RET immunostaining is not helpful to distinguish MEN 2-associated from sporadic phaeochromocytomas since the latter may also harbour gain-of-function *RET* mutations with consequent RET overexpression {1430}. In contrast to MEN 2-associated tumours, which harbour germline RET alterations, the *RET* mutations in sporadic tumours are somatic in nature {1131}.

Most series report a very low malignancy rate for phaeochromocytomas in MEN 2 patients when compared to sporadic tumours, but metastasizing tumours do occur {370,697,888}. In two series of 100 and 300 MEN 2 patients with phaeochromocytomas, a frequency of 3% and 4%, respectively, of malignant tumours was reported {324,1521}.

As in sporadic tumours, histological criteria to predict malignancy in tumours without metastases are not reliable in all cases and unequivocal immunohistochemical or molecular markers of malignancy are not yet available {1974,2229}. Malignant tumours are usually heavier, show coarse nodularity, confluent necrosis, absence of hyaline globules, higher mitotic rates, small cell morphology, reduced numbers or absence of sustentacular cells and may exhibit a different immunohistochemical expression pattern than benign tumours {392,469,790,1909, 1910,1979,2281,2289}.

In addition to phaeochromocytomas, so called composite phaeochromocytomas (with additional components of neuroblastoma, or ganglioneuroma, or ganglioneuroblastoma) arising in the adrenal medulla of MEN 2 patients have been described {238,1431}.

Prognosis and prognostic factors

Phaeochromocytoma as part of MEN 2 is almost always benign, with less than 5% reported to be malignant {324,1521}. Hence, the prognosis of patients with MEN 2 is mainly determined by the clinical course of their MTC. However, patients with phaeochromocytoma harbour a high risk of developing a hypertensive crisis, which may be lethal, during operations or childbirth. Hence, the presence of phaeochromocytoma needs to be excluded prior to a surgical procedure for MTC. If present, phaeochromocytoma has to be treated first.

Hyperparathyroidism

Clinical features

The symptoms of primary hyperparathyroidism (pHPT) are very well known as 'moans, groans, stones and bones'. In the 21st century, in countries where clinical surveillance and genetic testing are routine, patients with MEN 2 rarely present with these signs and symptoms. In comparison to sporadic pHPT and pHPT related to MEN 1, the disease appears to be rather mild and is generally diagnosed during follow-up.

The diagnosis is made based on the copresence of elevated parathyroid hormone and elevated serum calcium levels. The risk of untreated pHPT is diverse. Severe osteoporosis and osteopenia followed by bone fractures may develop. Besides kidney stones, patients may present with abdominal pain due to peptic ulcers and/or pancreatitis. Further, fatigue and lethargy may be

Roxane Labs., Inc.
Exhibit 1012
Page 045

present in the case of severe pHPT.
Prior to primary surgery, no preoperative imaging techniques are necessary. In the case of persistent or recurrent pHPT, however, sestamibi scanning, having a high sensitivity (90%) and specificity (almost 100%), is the preferred imaging technique to identify the responsible parathyroid tissue {2364}.

Pathology

The gross findings of parathyroid hyperplasia in MEN 2 do not differ from those of hyperplasia in a non-familial association. All four glands are generally enlarged and hypercellular with a relative paucity of intraparenchymal fat (classic or usual type hyperplasia). There can be considerable variation in the size of individual glands from a given case, as well as variation in the degree of intraparenchymal adipose tissue. Occasionally, one may find two glands that are markedly enlarged and two glands of near normal size (pseudoadenomatous hyperplasia), which does not preclude the diagnosis of parathyroid hyperplasia particularly in the setting of MEN 2. In a subset of cases, there is little variation from normal parathyroid glands grossly or histologically, with an abundance of intraparenchymal fat in all glands examined (occult hyperplasia) {196,1892}. Regardless of the disparity of size or degree of cellularity between one or more parathyroid glands from a patient with known MEN 2 the diagnosis of parathyroid adenoma should not be used.
Generally, the histological findings are the same as those described for hyperplasia occurring in a non-familial setting {478}. The predominant cell type is the chief cell, which has faintly eosinophilic cytoplasm and a centrally placed round relatively monotonous nucleus without conspicuous nucleoli. There is generally some variation in the amount of cytoplasm present, which often occurs in discrete foci creating a slightly nodular appearance at low power magnification. Some chief cells have more abundant cytoplasm with a lesser degree of eosinophilia that results in varying degrees of cytoplasmic clearing and are referred to as clear cells. This is not to be confused with diffuse water-clear („wasserhelle") cell hyperplasia, which is generally not associated with MEN 2 {478}. Oxyphil cells are the result of an increased number of mitochondria in the cytoplasm that results in a granular eosinophilic appearance. Regardless of the variation in cytoplasmic features the nuclear characteristics are remarkably similar among all cell types, although as in other endocrine organs, it is not uncommon to see focal nuclear atypia in benign parathyroid neoplasia. While nuclear atypia is more frequent in adenomas than hyperplasia {1245}, it is not uncommon to see nuclear enlargement with some degree of hyperchromasia or occasional multinucleated cells in parathyroid hyperplasia. Rarely does one see diffuse atypical nuclear changes from an MEN 2 family with particularly aggressive parathyroid disease.
The architectural pattern in all cases of parathyroid hyperplasia consists of cords or nests or cells in a glandular pattern as well as foci of solid sheets of cells (nodular hyperplasia), although these findings are not specific for MEN 2 and can be seen in parathyroid adenoma as well as non-familial hyperplasia. Occasionally the overall architectural pattern is predominantly follicle-like with a single or few rows of chief cells surrounding a centrally located pseudolumen that generally contains a slight amount of eosinophilic debris. The intraparenchymal fat content is generally reduced in parathyroid hyperplasia, but there is a great deal of variation in this finding.
The presence of mitotic figures in the absence of malignancy is a frequent finding in benign parathyroid neoplasia {1924} and this applies equally well in MEN 2 associated parathyroid hyperplasia {2073}.

Prognosis and prognostic factors

MEN 2A-related primary hyperparathyroidism is generally mild, however, single cases with severe forms have been observed.

Other component features of MEN 2

Hirschsprung disease

Hirschsprung disease (HSCR) (MIM #142623) is a congenital abnormality characterised by absence or hypoplasia of neurons and ganglia of the submucosal and myenteric plexuses along variable lengths of the hindgut {1698}. Loss-of-function mutations of the *RET* proto-oncogene may be the single most common cause of HSCR {544}. Co-segregation of MEN 2 and HSCR has been recognised in a small subset of MEN 2 cases (<1%) {1533,2173}. In these cases, both disease phenotypes arise from a single mutation of *RET*, generally affecting the extracellular cysteine codons 609, 611, 618 or 620. Although the mutant protein is constitutively active, reduced levels are expressed on the cell surface, leading to both loss-of-function (HSCR) and gain-of-function (MEN 2) phenotypes associated with a single *RET* mutation {1553,2173}.

Cutaneous lichen amyloidosis

In a few families with MEN 2, cutaneous lichen amyloidosis has been described {514,1623}. In these instances, cutaneous lichen amyloidosis has been assumed to be a paracrinopathy {1151}.

Genetics

MEN 2 is inherited as an autosomal dominant disease, caused by germline mutations of the *RET* (REarranged during Transfection) proto-oncogene.

Chromosomal location

All three disease phenotypes have been mapped to a single locus on chromosome 10q11.2 by linkage analyses, and *RET* mutations have been identified in each disease subtype {512,564,1556}.

Gene structure

The *RET* gene spans 21 exons, and encodes a transmembrane receptor tyrosine kinase with three protein isoforms of 1072, 1106 and 1114 amino acids that differ in their C-terminal residues {1566, 1697,2172}. All RET receptors have a large extracellular domain, involved in recognition and binding of RET-ligands and co-receptors, a transmembrane domain, and an intracellular tyrosine kinase domain which is required for autophosphorylation of the receptor and initiation of downstream signalling.

Gene expression

The RET tyrosine kinase is essential for the development of the kidney, central and peripheral nervous systems, and some neuroendocrine tissues, as well as for maturation of spermatogonia {102, 533,1485,1669}. Its expression is highest in neural crest cells and neural crest

Roxane Labs., Inc.
Exhibit 1012
Page 046

Table 5.02
Genotype-phenotype correlation in MEN 2.

Exon	Codon	FMTC	MEN 2A	MEN 2B
10	609	609	609	
	611	611	611	
	618	618	618	
	620	620	620	
11	630	630		
	634	634	634	
13	768	768		
	790	790	790	
	791	791	791	
14	804	804	804#	804†
	844	844		
15	883			883
	891	891	891¶	
16	918			918
Mean age at diagnosis§ (yrs)		45-55	25-35	10-20
Medullary thyroid carcinoma (MTC)		90-100%*	90-100%*	100%
Phaechromocytoma		-	40-60%	40-60%
Primary hyperparathyroidism		-	10-30%	-
Ganglioneuromatosis		-	-	+
Multiple mucosal neuromas		-	-	+
Marfanoid habitus		-	-	+
Thickened corneal fibers		-	-	+

§ The age at diagnosis has become younger since the identification of RET
# based on one report with MTC and adrenal and extra-adrenal phaeochromocytoma {1610}
† based on several reports with additional germline *RET* mutation {1046,1486,1516}; however, it appears that the phenotype is rather MEN 2B-like than typical MEN 2B
¶ based on one personal communication
* since the identification of *RET*, many patients undergo surgery before MTC occurs
– = disease/finding absent or frequency observed not higher that in the general population
+ = disease/finding present in most cases but neither required nor pathognomonic {742,746,1778}.

derived structures during embryogenesis and in the earliest stages of kidney induction and morphogenesis, but decreases to low levels in adult tissues {102,533,1669}.

Gene function

RET plays a role in development and maturation of peripheral nerves and in kidney induction but is also an important mechanism of neuronal survival in the peripheral nervous system, and particularly in the enteric nerve plexuses {226}. In normal cells, RET is activated by interaction with a multicomponent complex including members of both a soluble ligand family, the glial cell line-derived neurotrophic factors (GDNF), and also a family of cell surface bound co-receptors, the GDNF family receptors α (GFRα) {1886}.

Mutation spectrum

Activating germline mutations of *RET* are identified in >95% of all MEN 2 cases {564}. These are generally missense mutations clustered in only a few codons of *RET*, and there are strong correlations of specific disease phenotypes with subsets of these changes. As *RET* acts as an oncogene, a second somatic mutation of that locus is generally not part of MEN 2 tumourigenesis.

Genotype vs phenotype

Mutations of cysteine residues in the extracellular domain of *RET* (codons 609, 611, 618, 620 and 634) are found in most patients with MEN 2A and are strongly linked to the presence of phaeochromocytoma and hyperparathyroidism (HPT) {564,1554}. These mutations result in replacement of a cysteine normally involved in intramolecular bonds, leaving an unpaired cysteine residue available to form intermolecular bonds with other RET receptors, leading to constitutive dimerization and activation of RET signalling in the absence of its ligands and co-receptors {93,231,1934}. Patients with FMTC may have germline mutations of the same cysteine codons or may alternatively have mutations of intracellular residues (768, 790-91, 804, 844, 891) {564,982,1696}. The latter variants appear to alter RET binding of ATP and are reportedly associated with later onset, less aggressive disease phenotypes. The MEN 2B phenotype is associated almost exclusively with mutations of codons 918 (>95% cases) or 883 in the intracellular domain of RET {564,568, 718,906}. These mutations appear to alter *RET* substrate specificity and may result in stimulation of aberrant downstream signals which leads to the broader and more severe phenotype associated with this disease subtype {2100}.

Genetic counselling

All of the MEN 2 subtypes are inherited in an autosomal dominant manner with age-related penetrance.

MEN 2A. Almost all (>98%) affected individuals have an affected parent. Penetrance is at least 70% by 70 years of age with MTC as the usual first manifestation {562,1554,1761,1985}. In MEN 2A (and FMTC), *RET* mutation analysis can be restricted to exon 10, 11, 13, 14, and 15 (Table 5.02) {179,208,512,905,1556}.

MEN 2B. About 40% of affected individuals have *de novo* mutations {1621}. Thyroid carcinoma and multiple mucosal neuromata occur in virtually all affected individuals {1540}. If MEN 2B is suspected, mutation analysis should include exon 16 (M918T, >95%) {306,568,906} and 15 (A883F, 2-3%) {718,2069}.

FMTC. The presence of two or more MTC individuals on the same side of the family with objective evidence against phaeochromocytoma and hyperparathyroidism suggests FMTC. Age related penetrance is likely lower than MEN 2A {562}. However, FMTC is a clinical diagnosis and mutation carriers must not forego surveillance for other features although it is tempting to postulate that *RET* 768 and 804 mutations only result in FMTC.

Since *RET* gene mutations can be identified in >98% of all MEN 2 families, DNA-based diagnostic testing is considered standard of medical care in many countries worldwide {241,2223}. Gene testing should be offered to at-risk children in MEN 2A/FMTC families by age 6

Roxane Labs., Inc.
Exhibit 1012
Page 047

[241,562]. For children at risk of MEN 2B, gene testing should be immediate, and certainly prior to age 2 [1259]. Prenatal testing is technically possible when a *RET* mutation has been identified in an affected family member.

Preventive measures

Prophylactic thyroidectomy

For those individuals who test positive for a *RET* mutation, a total prophylactic thyroidectomy is recommended for all subtypes and is safe for all ages. This should be done prior to age 6 for MEN 2A and by the age of 6 months, certainly before 2 years of age, for MEN 2B [192,241, 526,1259,1308,1595,2374]. Surgical intervention at an early age may be less imperative in the codon 768 and 804 mutation families due to the lower penetrance and later onset of the thyroid disease [562,2022], however, clinical variability has been reported [613,666, 1259].

Screening recommendations following surgery depends on the pathology. If an individual has overt MTC at the time of surgery, screening should include calcium- or pentagastrin-stimulated calcitonin testing every 3-6 months for the first 2 years, every 6 months from 3-5 years after surgery, and then annually. If only a small focus of MTC is found at the time of surgery, follow-up screening should involve annual basal (unstimulated) calcitonin for 5-10 years. If no cancer is present in the thyroid, no follow-up screening is indicated, even if C-cell hyperplasia is present. All individuals who have undergone thyroidectomy need thyroid hormone replacement therapy and monitoring [241,562,716].

Screening for phaeochromocytoma

In MEN 2, phaeochromocytomas rarely occur before the presence of MTC [324]. However, at-risk individuals should undergo yearly screening for phaeochromocytoma, beginning at age 6. This consists of abdominal ultrasound or CT scans and 24-hour urine studies (vanillyl mandelic acid, metanephrines and catecholamines) [550]. Annual blood tests for chromogranin A and free catecholamine levels may be considered. Phaeochromocytoma should always be excluded in at-risk individuals prior to any surgery to avoid a life-threatening hypertensive crisis [241,562,716].

Screening for parathyroid adenoma or hyperplasia

Screening for parathyroid hyperplasia in MEN 2A includes annual blood tests measuring ionized calcium and intact parathyroid hormone (iPTH) levels beginning at the time of MEN 2 diagnosis. Once hyperplasia is diagnosed, surgery to remove the parathyroid glands is necessary. At that time or when thyroidectomy is performed, whichever occurs first, all 4 parathyroid glands and the thymus should be removed. Half of gland should be pulverize grafted into a muscle in the to control the body's calciu can be easily removed [241,562,716,2373]. Since involvement in MEN 2B is ra parathyroid screening is recommended unless othe ed [562].

RET mutation negative fam

For *RET* mutation negative risk individuals should unc screening for MTC, phaeocl and parathyroid hyperpla age of 6 to the age of 35 ye Prophylactic thyroidectomy routinely offered to this sub

Multiple endocrine neoplasia type 1

A. Calender
C.D. Morrison
P. Komminoth
J.Y. Scoazec
K.M. Sweet
B.T. Teh

Definition

Multiple endocrine neoplasia type 1 (MEN 1) is an autosomal dominant disease characterised by multifocal endocrine tumours affecting parathyroids, endocrine pancreas, anterior pituitary, cortical areas of the adrenal glands, diffuse endocrine tissues in thymus, bronchial tubes and various uncommon tumoural lesions in the skin, central nervous system and soft tissues.

MIM No. 131100 {1468}

Synonyms

The following terms should no longer be used: MEN I, Wermer syndrome, multiple endocrine adenomatosis type 1, familial Zollinger-Ellison syndrome.

Incidence

The prevalence in most populations is estimated to be between 1:40000 and 1:20000. About 10% of the patients have germline *MEN1* mutations arising de novo without any previous familial history {130}. Germline *MEN1* mutations have been found in about 5% of patients with sporadic primary hyperparathyroidism, a common disease occurring with a prevalence of 1:2000 to 1:1000 {2277}. Nevertheless, MEN 1 prevalence might be underestimated based on the observation that most patients predisposed to MEN 1 share at initial diagnosis a single endocrine lesion {2034}.

Table 5.03
Diagnostic criteria for MEN 1.

The presence of two or more of the following signs identifies the MEN 1 patient:

1. Primary hyperparathyroidism with multiglandular hyperplasia and/or adenoma or recurrent primary hyperparathyroidism
2. Duodenal and/or pancreatic endocrine tumours, both functioning (gastrinoma, insulinoma, glucagonoma) and non-functioning or multisecreting tumours as proven by immunohistochemistry, gastric enterochromaffin-like tumours
3. Anterior pituitary adenoma, both functioning (GH-secreting tumours or acromegaly, prolactinoma) and non-functioning or multisecreting (GH, PRL-prolactin, LH-FSH, TSH) lesions as proven by immunohistochemistry
4. Adrenocortical tumours, both functional and non-functioning
5. Thymic and/or bronchial tubes endocrine tumours (foregut carcinoids)
6. A first-degree relative (parent, sibling, or offspring) with MEN 1 according the above criteria.

Diagnostic criteria

The diagnosis of MEN 1 should be suggested in individuals with newly diagnosed endocrine neoplasia component of MEN 1 (e.g., primary hyperparathyroidism, gastroenteropancreatic tumour, pituitary adenoma, adrenocortical and thymic endocrine tumours). This diagnosis is further strengthened if additional criteria commonly related to inherited cancers are met (e.g., age <50 years; positive family history; multifocal or recurrent neoplasia; two or more endocrine organs or systems affected). The diagnostic criteria for MEN 1 are given in Table 5.03.

Hyperparathyroidism

Age, distribution and penetrance

MEN 1 has a very high penetrance and an equal sex distribution. Although rare in early teens or younger, the cumulative percentages of patients who develop biochemical evidence of hyperparathyroidism increase with age. They reach 43%, 85%, and 94% at the ages of 20, 35, 50 years, respectively {2269}.

Clinical features

MEN 1-related parathyroid disease manifests as a multiglandular disorder rather than solitary adenoma, the latter of which is a common feature in sporadic primary hyperparathyroidism. As a result, recurrence is common in patients who only have partial parathyroidectomy. Parathyroid carcinoma, a feature of familial hyperparathyroidism-jaw tumour syndrome, however, is not known to be associated with MEN 1 and shown related to the HRPT2 locus on chromosome 1 {319}. Although "moans, groans and stones" are the hallmarks of severe or longstanding hypercalcemia, the majority of MEN 1 cases are asymptomatic and commonly detected through routine biochemical investigations.

Pathology

The gross findings of parathyroid hyperplasia in MEN 1 do not differ from those of hyperplasia in a non-familial association. All four glands are generally enlarged and hypercellular with a relative paucity of intraparenchymal fat (classic or usual type hyperplasia). There can be considerable variation in the size of individual glands from a given case, as well as variation in the degree of intraparenchymal adipose tissue. Occasionally, one may find two glands that are markedly enlarged and two glands of near normal size (pseudoadenomatous hyperplasia), which does not preclude the diagnosis of parathyroid hyperplasia particularly in the setting of MEN 1. In a subset of cases there is little variation from normal parathyroid glands grossly or histologically, with an abundance of intraparenchymal fat in all glands examined (occult hyperplasia) {196,1892}. Regardless of the disparity in size or degree of cellularity between one or more parathyroid glands from a patient with known MEN 1, the diagnosis of parathyroid ade-

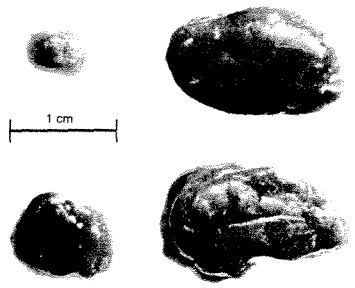

Fig. 5.06 Parathyroid hyperplasia. Macroscopic aspect of the parathyroid glands of a normal individual (left) and a MEN1 patient (right).

Roxane Labs., Inc.
Exhibit 1012
Page 049

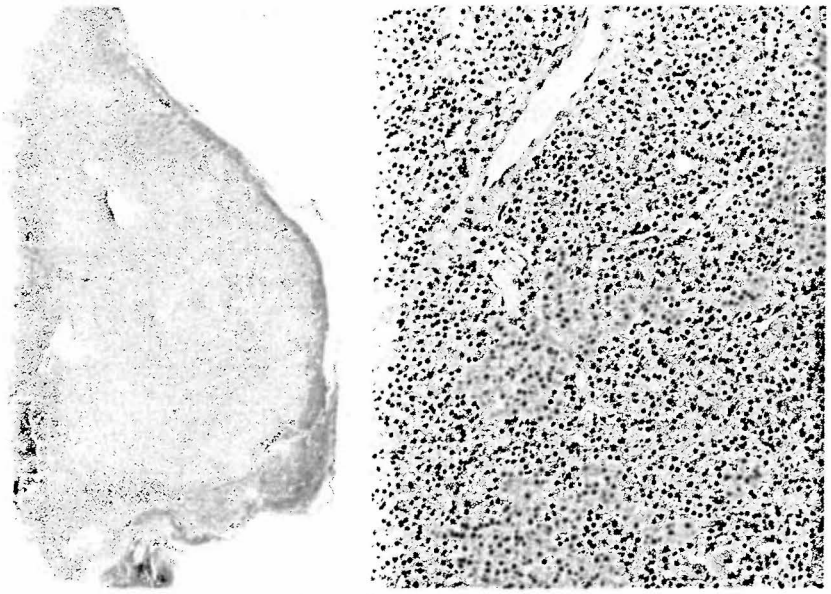

Fig. 5.07 Parathyroid hyperplasia. Gross section and histopathological features of a parathyroid gland of a MEN1 patient.

noma should not be used.

Generally, the histological findings are the same as those described for hyperplasia occurring in a non-familial setting {478}. The predominant cell type is the chief cell, which has faintly eosinophilic cytoplasm and a centrally placed round relatively monotonous nucleus without conspicuous nucleoli. There is generally some variation in the amount of cytoplasm present, which often occurs in discrete foci creating a slightly nodular appearance at low power magnification. Some chief cells have more abundant cytoplasm with a lesser degree of eosinophilia that results in varying degrees of cytoplasmic clearing. This is not to be confused with diffuse water-clear (wasserhelle) cell hyperplasia, which is generally not associated with MEN 1 {1245}. Oxyphil cells are the result of an increased number of mitochondria in the cytoplasm that results in a granular eosinophilic appearance. Regardless of the variation in cytoplasmic features, the nuclear characteristics are remarkably similar among all cell types, although, as in other endocrine organs, it is not uncommon to see focal nuclear atypia in benign parathyroid neoplasia. While nuclear atypia is more frequent in adenomas than hyperplasia {1245}, it is not uncommon to see nuclear enlargement with some degree of hyperchromasia or occasional multinucleated cells in parathyroid hyperplasia.

The architectural pattern in all cases of parathyroid hyperplasia consists of cords or nests or cells in a glandular pattern as well as foci of solid sheets of cells (nodular hyperplasia). These findings are not specific for MEN 1 and can be seen in parathyroid adenoma as well as non-familial hyperplasia. Occasionally, the overall architectural pattern is predominantly follicle-like with a single or few rows of chief cells surrounding a centrally located pseudolumen that generally contains a slight amount of eosinophilic debris. The intraparenchymal fat content is generally reduced in parathyroid hyperplasia, but there is a great deal of variation in this finding.

The presence of mitotic figures in the absence of malignancy is a frequent finding in benign parathyroid neoplasia {1923} and this applies equally well in MEN 1 associated parathyroid hyperplasia {2073}.

Prognosis and prognostic factors

The patients are treated by surgery, either a total parathyroidectomy with autotransplantation or subtotal resection of three and a half parathyroid glands. The prognosis for these patients after such treatment should be no different from sporadic counterparts. However, because of the genetic basis of their parathyroid disease, half glands left behind in the neck or autotransplated glands are still at risk for rehyperplasia. These patients are believed not to develop malignancy.

Pituitary tumours

Clinical features

Anterior pituitary adenoma is the first clinical manifestation of MEN 1 in around 10% and 20% of familial and sporadic cases, respectively {322,2310}. Its prevalence in MEN 1 varies from 20-60% with a mean estimate value of 42% in largest studies. Mean age of onset of pituitary tumours is 38 ±15 yr with rare occurrences in children younger than 5 yrs {2149}. Of the various subtypes of MEN 1-related pituitary adenomas, prolactinomas (PRL) are the most commonly seen (≈60%), followed by growth hormome (GH) secreting (≈10%), adrenocorticotropin (ACTH) secreting (≈5%) and nonsecreting (≈15%) adenomas. Around 10% of MEN 1 pituitary adenomas show cosecreting immunohistochemical reactivities including PRL, GH, ACTH and less commonly LH, FSH and TSH. More than 80% of MEN 1-related pituitary lesions are macroadenomas including 30-35% of invasive cases. MEN 1 pituitary adenomas are significantly more frequent in women than in men (50% versus 30%) the easier recognition of clinical signs related to an excess of prolactin secretion in women may account for this sex-ratio. When present, major clinical signs are those observed in sporadic counterparts of these tumours: amenorrhea, infertility, galactorrhea in women, hypogonadism in

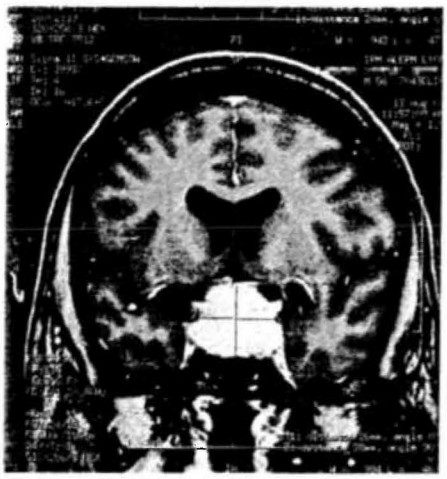

Fig. 5.08 MEN 1. Frontal magnetic resonance imaging showing a MEN1-related pituitary macroadenoma.

Roxane Labs., Inc.
Exhibit 1012
Page 050

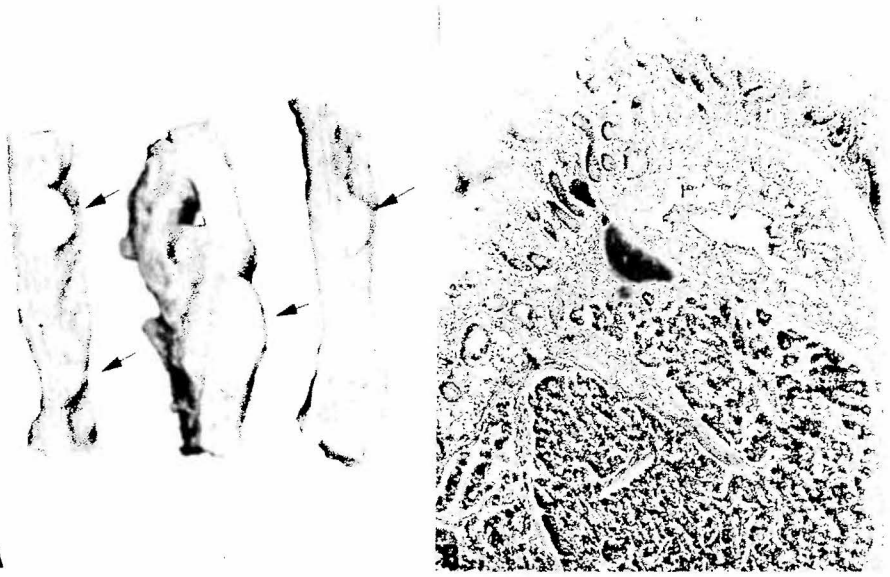

Fig. 5.09 Multiple endocrine neoplasia type 1 (MEN 1). **A** Multiple endocrine tumours in the mucosa/submucosa (see arrows) of a MEN1 patient suffering from Zollinger Ellison syndrome. **B** Well differentiated endocrine carinoma in a submucosa of the duodenum in a patient with MEN1 with Zollinger-Ellison syndrome.

men, related to prolactinomas, acromegaly secondary to excess GH and Cushing disease related to overproduction of ACTH and secondary adrenal hyperplasia. About one-third of tumours are invasive macroadenomas {2310} which can cause morbidity due to mass effects, leading to hypopituitarism and compression of adjacent structures, especially of the optic chiasm. The diagnosis of MEN 1 pituitary tumours requires a careful clinical history, including family history, basal hormone (PRL, GH, IGF-1) levels and radiological screening of the pituitary gland by magnetic resonance imaging.

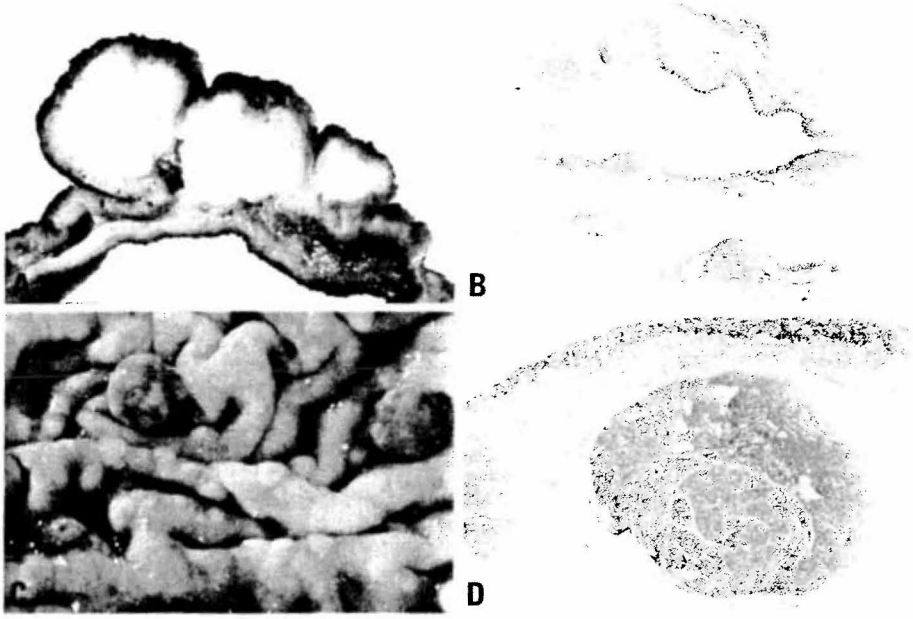

Fig. 5.10 Multiple endocrine neoplasia type 1 (MEN 1). **A** Cut surface of the wall of the stomach from a patient with MEN1 and Zollinger Ellison syndrome, showing multiple endocrine tumours in the mucosa and submucosa (ECLomas). **B** Microscopic sections of the duodenum from a MEN1 patient. In the mucosa and submucosa there are multiple endocrine tumours (gastrinomas). **C** Microcarcinoidosis of the stomach. **D** Two adjacent gastrinomas in the mucosa/submucosa of a MEN1 patient.

Pathology

The majority of MEN 1 patients wi involvement of the anterior pituita exhibit a single adenoma and multice tricity appears to be extremely ra {1033,2044}. Convincing evidence fo isolated diffuse hyperplasia of one of th cell types, similar to findings in th parathyroid and endocrine pancreas MEN 1 patients, has not yet been repor ed; however, PRL or GH cell hyperplas of the peritumoural parenchyma ha been described {295,1626}.

In general, all types of adenomas an immunohistochemical expression pa terns may be encountered in MEN patients and the morphological feature are not different from those seen in sp radic pituitary adenomas. However, i several series, a higher frequency o functionally active tumours with a hig proportion of prolactinomas was repor ed when compared to sporadic pituita adenomas {295}. A substantial propo tion of tumours are microadenomas (<1 mm), but in one series from 1987, thric the frequency of macroadenomas (≥1 mm) was reported {1967}. Immuno histochemically, approximately one thir of adenomas produce more than on hormone and the majority exhibit a pos tive prolactin immunostaining (73-76%) followed by growth hormone (14–37%) o both {295,1960,1960}. Tumours expres ing ACTH or other hormones {2426} ar less frequent and non-functioning aden mas are rare.

Prognosis and prognostic factors

Treatment of pituitary tumours in MEN varies according to the type and stagin of the adenoma and is identical to tha applied in sporadic counterparts. Ther is no good data to suggest that prognos tic outcome is any different from the sp radic counterpart. However, becaus clinical surveillance may be routine, th MEN 1-related pituitary tumours may b detected at an earlier stage {412A,2310}

Duodenal and pancreatic endocrine tumours

Clinical features

Endocrine tumours of the duodenum an pancreas are common manifestations i MEN 1 disease. Clinical expression o these tumours is highly variable an related to the extent, localisation, natur

Roxane Labs., Inc.
Exhibit 1012
Page 051

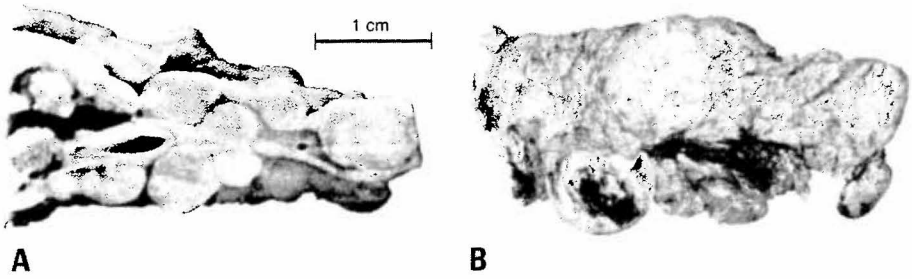

Fig. 5.11 Multiple endocrine neoplasia type 1 (MEN 1). **A** Adrenal nodular hyperplasia. **B** Resection specimen from the tail of the pancreas from a patient with MEN1. The cut surface shows three well demarcated tumours.

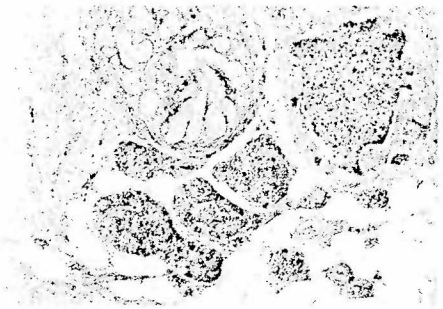

Fig. 5.12 Multiple endocrine neoplasia type 1 (MEN 1). Microadenoma (upper right) adjacent to a group of islets.

and level of hormone(s) secreted by tumoural cells. Predominant hormone secretion may occur and leads to a specific clinical syndrome.

Zollinger-Ellison syndrome (ZES)

This is the most frequent clinical state related to duodenal and/or pancreatic gastrinoma observed in MEN 1 patients and due to excessive production of gastrin by tumour cells. Initial symptoms are caused by gastric acid hypersecretion {556}. Abdominal pain or gastro-oesophageal reflux disease or both can occur in up to 80% of patients, while diarrhoea occurs in 10-20%. Atypical peptic ulcer disease with multiple lesions or ulcers in unusual locations should suggest the diagnosis of MEN 1-related ZES {1003}. Gastrinomas occur in 20-60% of MEN 1 patients and management of gastric acid secretion by H^+-K^+ ATPase inhibitors (proton pump inhibitors) has significantly reduced or abrogated the risk of severe complications of ZES such as bleeding, perforation and esophageal strictures. In about 90% of MEN 1 patients with ZES, the source of gastrin excess is usually multiple small duodenal tumours. Diagnosis of gastrinomas includes measurement of specific biological and hormonal levels, such as fasting gastrin levels, measurement of gastric pH and gastrin provocative secretin test {668,1003}.

The early diagnosis of pancreatic endocrine tumours is enhanced by a standardised meal stimulation test and further measurement of serum gastrin and pancreatic polypeptide (PP) {2066}. Radiological imagine of the upper abdomen are performed every third year in *MEN1* gene carriers and include percutaneous and endoscopic echography, spiral computed tomography (CT), and somatostatin receptor (octreotide) scintigraphy.

Insulinomas

These are the second most frequent pancreatic tumours observed in the setting of MEN 1. Among large series of patients tested, one would expect that about 5-10% of patients with insulinomas belong to a MEN 1 genetic background {2012}. Common and convincing symptoms result from hypoglycaemia, followed by a catecholamine response. The mean duration of symptoms in patients without previous clinical history before diagnosis has ranged from 1 year to 3-5 years and early recognition of *MEN1* gene carriers may now avoid long delays in insulinoma recognition. Various short and long term fasting tests and concomitant determinations of serum glucose and insulin levels are the most reliable biological procedures for insulinoma diagnosis. Apart from standard abdominal radiological examinations such as CT and ultrasonography, specific techniques for tumour localization including arteriography, trans-hepatic portal venous sampling, selective arterial calcium stimulation with hepatic venous sampling and intraoperative ultrasonography have been shown to represent reliable procedures {1620}.

Glucagonoma, VIPoma and other uncommon pancreatic endocrine tumours occur in less than 5% of MEN 1 patients but are characterised by a high malignant potential {1000}. More than 70% of glucagonomas and 40% VIPomas are malignant. Glucagonomas induce a necrolytic migratory erythema associated with diabetes mellitus secondary to abnormal glucagon secretion, VIPomas clinical expression is related to excessive production of vasoactive intestinal pep-

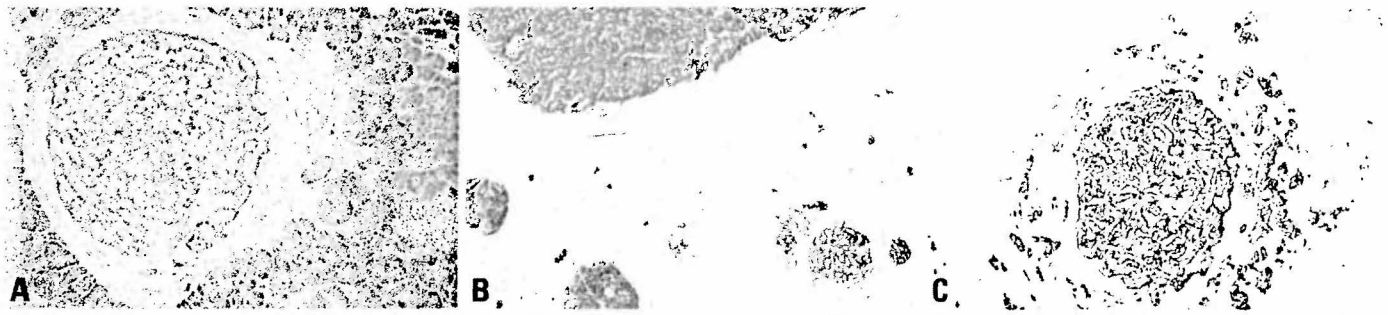

Fig. 5.13 Multiple endocrine neoplasia type 1 (MEN 1). **A** Microadenoma (< 5 mm) in a MEN1 patient. In comparison note the normal islet on the right. **B** Synaptophysin immunostaining exhibits parts of an encapsulated macrotumour (top) as well as several microadenomas. Same case as figure A. **C** Pancreatic microadenoma immunostained for glucagon.

Roxane Labs., Inc.
Exhibit 1012
Page 052

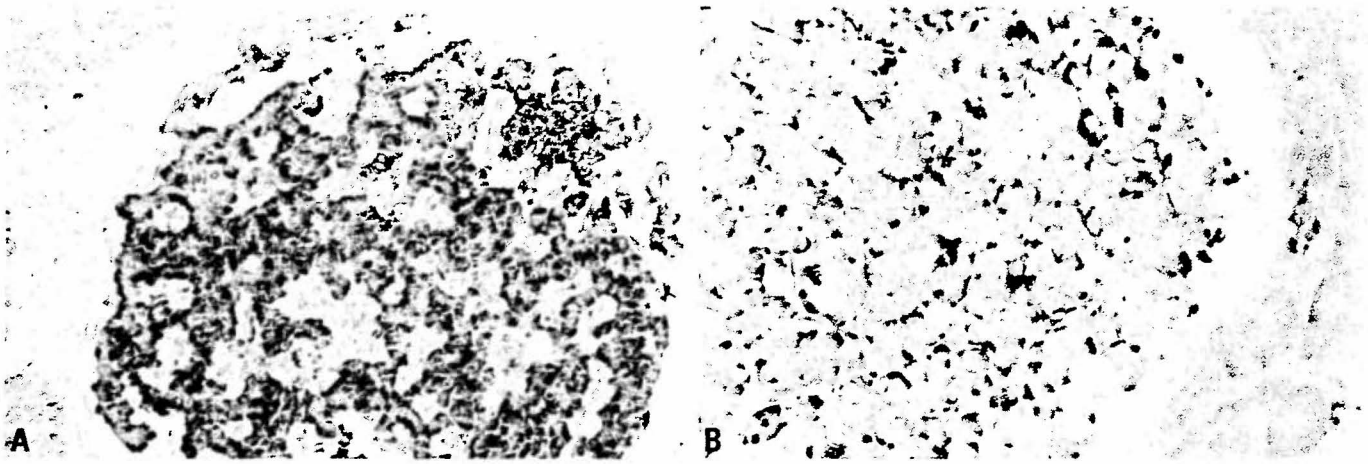

Fig. 5.14 Multiple endocrine neoplasia type 1 (MEN 1). **A** Pancreatic microadenoma exhibiting PP immunoreactive cells. **B** MEN1 microadenoma exhibiting cells immunoreactive for glucagon. [Immunostains using DAB as chromogen, followed by nickle-cobalt intensification and nuclear red as counterstain.]

tide (VIP) with the classical Verner-Morrison syndrome associating watery diarrhoea, hypokaliemia and achlorhydria (WDHA) {354,1691}. Very rare tumours observed in MEN 1 or a sporadic context are somatostatinoma and those secreting ACTH, calcitonin or GRF (Growth Releasing factors). Radiological diagnosis is performed with previously described methods.

Non-functional pancreatic endocrine tumours are characterised by the absence of any symptoms related to hormone hypersecretion. Nevertheless, they may have in situ production of hormones and peptides without biological effects (NSE) {1250}. Fortuitous diagnosis of non-functioning tumours is common mainly in the context of MEN 1 clinical surveillance protocols. Measurement of serum insulin, proinsulin, PP, glucagon and gastrin both in basal and provocative conditions may be useful, but repeated assessment of of serum chromogranin A (CgA) levels is considered more accurate (about 70% senstivity) for an earlier diagnosis of these insidious tumours {135}. Non-functioning pancreatic endocrine tumours may occur in 20-40% of MEN 1 patients {1753}. When misdiagnosed, they are often discovered after local compression and/or hepatic metastases occur.

Pathology

Pancreatic involvement in MEN 1 patients occurs in 30-75% of patients when assessed by clinical screening methods and approaches 100% in autopsy series {1388}. The majority of affected patients exhibit numerous non-functioning microadenomas (<0.5 cm) spread throughout the pancreas {1114} with a predominance of the pancreatic tail {881}. The small tumours usually display a distinct trabecular pattern and may show conspicuous connective tissue stroma {1114}. Immunohistochemically, they express one or several hormones and usually stain for pancreatic polypeptide, glucagon and/or insulin, but are functionally silent {229,1114,1741}. At the time of diagnosis, larger endocrine pancreatic tumours are usually multiple, vary in size {1950} and may be cystic {1301}. Clinically they can secret different hormones. Hyperinsulinism is seen in about one fourth of patients. Histologically they show a solid, adenomatous or trabecular pattern. Tumours with amyloid deposition usually exhibit insulin production. Similar to pituitary lesions, endocrine pancreatic tumours in MEN 1

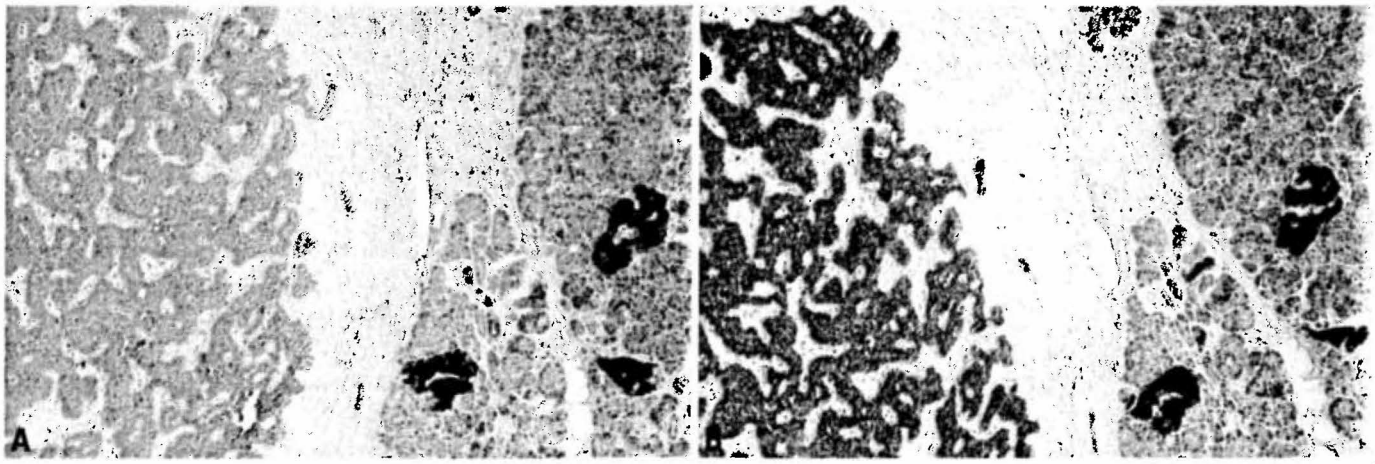

Fig. 5.15 Multiple endocrine neoplasia type 1 (MEN 1). **A** Menin (C-terminal epitope) immunohistochemistry in a MEN1-associated pancreatic endocrine tumour (left). Note the weak immunohistochemical signal in the tumour compared to the strong signal in the adjacent islets. **B** Menin (N-terminal epitope) immunohistochemistry in a MEN 1 associated pancreatic endocrine tumour. Note the retained immunohistochemical signal in the tumour and the strong signal in the adjacent islets.

Roxane Labs., Inc.
Exhibit 1012
Page 053

patients consistently express multiple hormones {2171} but one hormone usually predominates {1111}. However, similar to sporadic endocrine pancreatic tumours, immunohistochemical expression patterns do not necessarily correlate with blood hormone levels or clinical syndromes due to possible impaired hormone secretion of tumour cells or the production of functionally inactive but immunoreactive molecules. Pancreatic polypeptide and glucagon containing tumours are most often found {1741} while tumours expressing predominantly or exclusively insulin are less common {1250}. Tumours with main immunoreactivity for somatostatin, vasointestinal polypeptide or other hormones are rare {173,552,1264,1741} and those with pure gastrin expression are an exception. The pancreatic tumours only rarely give rise to metastases and criteria to define malignancy are the same as for sporadic tumours. As for other endocrine tumours no reliable markers of malignancy are available {1214,1836}. In addition to microadenomas and endocrine macrotumours, the pancreas' of MEN 1 patients frequently exhibit small nests of endocrine cell budding from ducts as well as ill shaped islet-like cell clusters with cellular irregularities and abnormal distribution of the four islet cell types. These nesidioblastosis-like features are mainly seen in cases with additional severe obstructive pancreatitis due to duct stenoses by large endocrine tumours and should therefore not be regarded as the precursor lesion of the pancreatic endocrine tumours in MEN 1 {1180}. The Zollinger-Ellison syndrome occurring in approximately 50% of MEN 1 patients is mainly due to functioning gastrinomas located in the duodenum and is only exceptionally associated with pancreatic tumours. The gastrinomas are mostly located in the mucosa and submucosa of the proximal duodenum, are multiple and very small (<1 cm) and thus difficult to localise clinically, at surgery or autopsy {515}. Immunocytochemically, they stain almost exclusively for gastrin. They may give rise to periduodenal-parapancreatic lymph node metastases, which can be found in 60-80% of the cases and may be much larger than the primary tumour {1748}. The development of liver metastases occurs late in the course of the disease. Multiple somatostatinomas of the duodenum have also been described in MEN 1 pancreas' but appear to be very rare {1037,2451}.

Fig. 5.16 Microscopic aspect of an angiofibroma in the skin of a MEN1 patient.

Prognosis and prognostic factors

Malignancy of duodenal and pancreatic endocrine tumours has been recognized for all subtypes and mainly for gastrinomas (≥40%), glucagonoma (≥80%), VIPoma (≥40%) and non-functional tumours (≥70%). Malignant insulinomas are less common and may represent 5-10% of patients. Cure is highly dependent of tumour size and early diagnosis in asymptomatic gene carriers is a prerequisite for medical and/or surgical treatment success. Long-term studies suggest that about 23% of MEN 1-related gastrinomas develop liver metastases and 14% demonstrate aggressive growth. After a mean follow-up of 8 years, 4% of patients had died {710}. In a large study of patients who underwent surgery, 34% of patients with sporadic gastrinomas were free of disease at 10 years, as compared with none of the patients with MEN 1 {1619}. The overall 10-year survival rate was 94%.

Other component features of MEN 1

Adrenal cortical lesions

Adrenal cortical lesions are observed in 20-40% of MEN 1 patients and commonly hyperplastic, bilateral and nonfunctional {2067}. Most of MEN 1 adrenal enlargements and tumours remain asymptomatic and are commonly diagnosed 5 years later than MEN 1 {1231}. Median tumour diameter at diagnosis is 3.0 cm, with most tumours being 3 cm or smaller. In rare cases, hyperaldosteronism and Cushing syndrome have been reported {148,1231}. Most tumours are non-functioning and about 15-20% of them become malignant. The lesions are often small and non-functioning and can therefore be managed by close surveillance but others have significant malignant potential and should be considered for surgery (subtotal adrenalectomy) when they are 3 cm or larger.

Cutaneous proliferations

Cutaneous lesions are present in 40-80% of MEN 1 patients, with variable histological forms {459}. Nodular lipomas are usually multicentric and show no recurrence after surgery. Large visceral lipomas have been also reported. The most common lesions are collagenomas and multiple and facial angiofibromas {459,1907}. Careful attention to cutaneous lesions in patients with endocrine tumours may be relevant for an earlier clinical diagnosis of MEN 1. Angiofibromas are clinically and histologically identical to those in individuals with tuberous sclerosis. Less common cutaneous lesions observed in MEN 1 are cafe au lait macules, confetti-like hypopigmented macules and multiple gingival papules in 2 patients {459}. Primary malignant melanomas have been observed in patients with MEN 1 belonging to large families {1615}. This highlights a possible role of the *MEN1* gene in the tumourigenesis of a small subgroup of melanomas and conversely suggests that MEN 1 patients have a slightly increased predisposition to cutaneous and uncommonly choroidal melanoma.

Thymic and bronchial neuroendocrine tumours

These tumours are observed in about 5-10% of MEN 1 patients. As for duodenal and pancreatic endocrine tumours, they originate in the foregut. Thymic neuroendocrine tumours (carcinoids) are observed predominantly in males and carry a poor prognosis {2209}. MEN 1-related thymic carcinoids constitute approximately 25% of all cases of thymic carcinoids. In patients with MEN 1, this is an insidious tumour not associated with Cushing or carcinoid syndromes {708, 2209}. Local invasion, recurrence, and distant metastasis are common, with no known effective treatment. Computed tomography or magnetic resonance imaging of the chest, as well as octreoscanning, should be considered as part of clinical screening in patients with MEN 1. Prophylactic thymectomy during sub-

Roxane Labs., Inc.
Exhibit 1012
Page 054