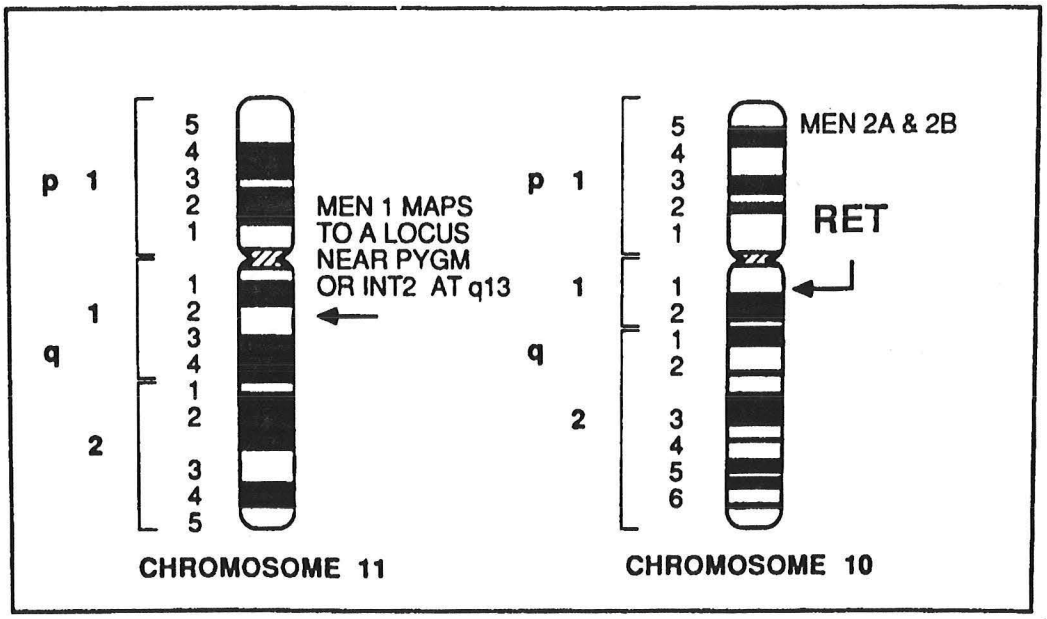
MULTIPLE ENDOCRINE NEOPLASIA



Kevin J. Petty, M.D., Ph.D.

University of Texas Southwestern Medical Center

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INTRODUCTION

Multiple endocrine neoplasia (MEN) is a term used to describe syndromes in which tumors (usually benign) develop in more than one endocrine organ with a familial pattern. The MEN syndromes were first recognized almost 100 years ago [1]. They are classified into two different types, MEN 1 (Wermer's syndrome) [2] and MEN 2 (Sipple's syndrome) [3]. MEN 2 is further subdivided into MEN 2A and MEN 2B. Multiple endocrine adenomatosis (MEA) is a now infrequently used term for these syndromes. The MEN syndromes have distinct clinical features, and they differ in their presentation and management. However, they do share certain features summarized in the following table.

FEATURES COMMON TO THE MEN SYNDROMES

1. **FAMILIAL (AUTOSOMAL DOMINANT)**
2. **HYPERPLASIA -> ADENOMA (USUALLY BENIGN)**
3. **MULTICENTRIC (POLYCLONAL) ORIGIN**

MULTIPLE ENDOCRINE NEOPLASIA TYPE 1 (MEN 1)

MEN 1 was first described in 1903 [1] but its genetic etiology was not recognized until 1954 [2]. The familial nature of MEN 1 follows an autosomal dominant pattern with high penetrance. Incidence of MEN 1 is difficult to determine but evidence of MEN 1 has been detected in as many as 0.25% of unselected autopsies [4]. The prevalence is estimated at 0.02 to 0.2 per 1000 persons but this probably underestimates the actual number because many cases go unrecognized [5]. Both sexes are affected equally, and the disease usually does not manifest itself prior to age 17, but becomes clinically apparent in most if not all affected individuals by age 40 [5-7].

The tumors that develop usually occur in the parathyroid glands, the anterior pituitary, and/or the pancreas (the three P's). The affected glands can contain different histologic processes (hyperplasia, adenoma, carcinoma), and a single tumor can produce several different hormones. It is notable that this is a disease with significant interfamilial variability but with intrafamilial uniformity. Other tumors that have been reported rarely in MEN 1 include lipoma, thymoma, and colon polyposis [8].

Endocrine gland involvement with multiple endocrine neoplasia type 1 (MEN 1)

Organ* (% involvement)	Pathology	Peptide	Clinical features
Pituitary (30–65)	Prolactinoma	PRL	Asymptomatic, infertility, galactorrhea
	Growth hormone	GH	Acromegaly, headache
	Cushing's disease	ACTH	Cushing's syndrome
	Nonfunctioning	? PRL	Asymptomatic, field defect
Parathyroid (80–90) (less often)	Hyperplasia Multicentric adenoma	PTH	Hypercalcemia
Pancreas† (40–85)	Gastrinoma	Gastrin	Zollinger–Ellison syndrome
	Insulinoma	Insulin	Hypoglycemia
	VIPoma	VIP	WDS (WDHA)
	Glucagonoma	Glucagon	Skin lesions, NIDDM
	GHRHoma	GHRH	Acromegaly, secondary GH release
	PPoma	PP	Asymptomatic, diarrhea (rarely)
Other (?)	Carcinoid	Substance P, Serotonin	Flushing, diarrhea

ACTH, adrenocorticotropic hormone; GH, growth hormone; GHRH, growth hormone-releasing hormone; MEN, multiple endocrine neoplasia; NIDDM, noninsulin-dependent diabetes mellitus; PP, pancreatic peptide; PRL, prolactin; PTH, parathyroid hormone; VIP, vasoactive intestinal peptide; WDS, watery diarrhea syndrome, also termed Verner–Morrison syndrome, pancreatic cholera or WDHA (watery diarrhea, hypokalemia, achlorhydria)

* Major organ involved.

† The two most common endocrine tumors of the pancreas are gastrinoma and insulinoma

MEN 1: Tumors

Parathyroid. Hyperparathyroidism is the most common manifestation of MEN 1 occurring in at least 90% of cases [9]. It is most often diagnosed by the presence of asymptomatic hypercalcemia with elevated serum PTH (primary hyperparathyroidism). Prospective screening of patients at risk identifies affected MEN 1 patients at an average age of 19 years (range 12-28) [5]. The earliest lesion is diffuse chief cell hyperplasia of all of the parathyroid glands. The hyperplasia is presumed to be the result of continuous stimulation of the glands by a circulating mitogenic factor that is closely related to basic fibroblast growth factor (bFGF) [10-14]. With time, adenomas develop that arise from the proliferation of a single cell clone [15].

The hyperparathyroidism of MEN 1 is slightly different from that of sporadic hyperparathyroidism in the early stages. Compared to sporadic hyperparathyroidism, the secretion of PTH from hyperplastic parathyroid glands in MEN 1 tends to be more responsive to serum calcium. Consequently, the hypercalcemia and hypophosphatemia of hyperparathyroidism in MEN 1 tend to be less pronounced than in sporadic hyperparathyroidism. Once an adenoma has developed, the hyperparathyroidism of MEN 1 is essentially indistinguishable clinically from sporadic hyperparathyroidism. This is reflected by the fact that the morbidity of hyperparathyroidism (nephrolithiasis, renal impairment, osteoporosis, mental status changes, bone pain) is the same in MEN 1 and non-MEN 1 cases [16]. However, peptic ulcer disease is more common in MEN 1 due to the presence of gastrinoma.

Hyperparathyroidism occurs in other familial syndromes that are unrelated to MEN 1. The genetic defects in these syndromes have not been identified.

Familial endocrinopathies with primary hyperparathyroidism

Dominant syndromes
MEN 1
MEN 2A
Familial cystic parathyroid adenomatosis with fibroosseous jaw tumors
Dominant isolated familial parathyroid adenomas
Dominant isolated familial parathyroid hyperplasia
Benign familial (hypocalciuric) hypercalcemia with hyperparathyroidism
Syndromes of recessive or uncertain inheritance
Recessive isolated familial parathyroid adenomas
Recessive isolated familial parathyroid hyperplasia
Familial parathyroid hyperplasia with colon polyps or carcinoma
Familial parathyroid hyperplasia with parathyroid carcinoma

MEN, multiple endocrine neoplasia.

Hereditary multiple endocrine neoplasia (MEN) syndromes with minimal or uncertain parathyroid involvement

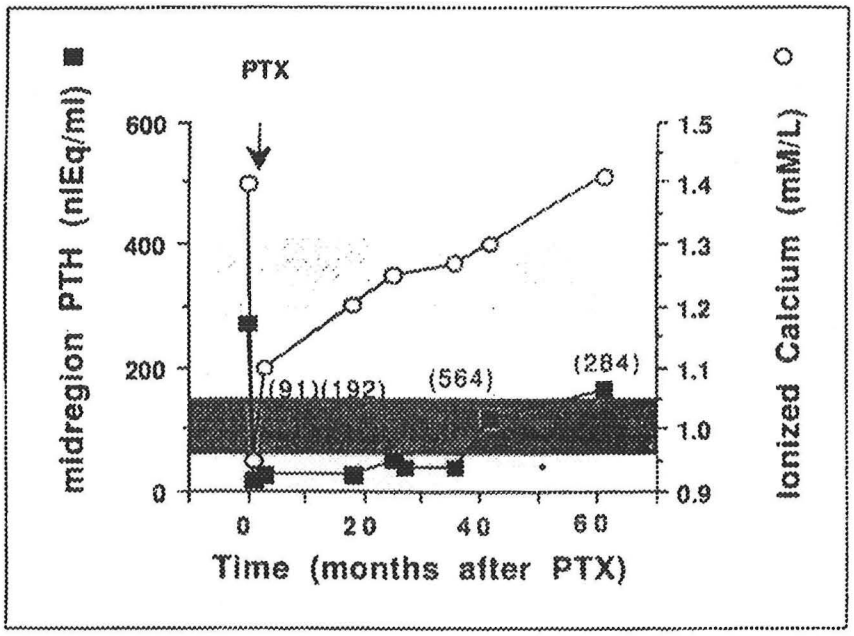
Syndromes of dominant inheritance
MEN 2 variants (map to chromosome 10)
MEN 2A with lichen amyloidosis
Familial medullary carcinoma (no pheochromocytoma or hyperparathyroidism)
MEN 2B
von Hippel–Lindau's disease (CNS hemangioblastomas, retinal angiomas, renal-cell carcinomas, pheochromocytomas, visceral cysts, and islet-cell tumors)
Familial pheochromocytoma (no thyroid, retinal, or CNS lesions)
Familial papillary thyroid carcinoma
Familial arrhenoblastoma and thyroid adenoma
Syndromes of recessive or uncertain inheritance
Myxomas, spotty pigmentation and nodular adrenal, testicular, or pituitary tumors
Gastric leiomyosarcoma, extraadrenal paraganglioma, pulmonary chondroma
Extraadrenal paragangliomas and pituitary adenoma (may include primary parathyroid hyperplasia and may have dominant inheritance with partial penetrance)

CNS, central nervous system.

It may be difficult to distinguish the hyperparathyroidism of MEN 1 from sporadic hyperparathyroidism based on clinical features alone because most MEN 1 patients may present initially only with hyperparathyroidism. In one series, as many as 28% of patients initially thought to have sporadic hyperparathyroidism were eventually found to have MEN 1 [17]. Hypercalcemia per se can stimulate gastrin secretion, and PTH can stimulate prolactin secretion [18, 19]. Thus the presence of hypergastrinemia or hyperprolactinemia in patients with hyperparathyroidism does not always prove the existence of MEN 1. The diagnosis of MEN 1 is strongly suggested by a positive family history for MEN 1, but family history might be unavailable or uninformative if the proband represents a new mutation. Currently, the diagnosis of MEN 1 should be considered in all patients with primary hyperparathyroidism, particularly those who have diffuse hyperplasia of the parathyroid glands (with or without adenoma) at the time of surgery.

Surgery is the treatment of choice for hyperparathyroidism in MEN 1. Most endocrine surgeons will locate and conduct a thorough examination of all of the parathyroid glands at the time of surgery. If MEN 1 is strongly suspected based on family history or the presence of other endocrine tumors in the patient, the surgical procedure of choice is a total parathyroidectomy

with reimplantation of approximately one-half of a minced parathyroid gland (150 mg) into the anterior, non-dominant forearm. The grafted tissue may take a few weeks to start functioning but the rate of permanent hypoparathyroidism is less than 1% [9]. Some endocrine surgeons also perform a transcervical thymectomy to be certain that potential ectopic thyroid tissue in the thymus is removed [20]. The hyperplastic parathyroid tissue in MEN 1 patients will continue to grow and hyperparathyroidism will recur in 50% within 10 years of the first surgery [7, 21]. Forearm reimplantation simplifies the second operation for hyperparathyroidism by allowing reexploration of the forearm under local anesthesia.

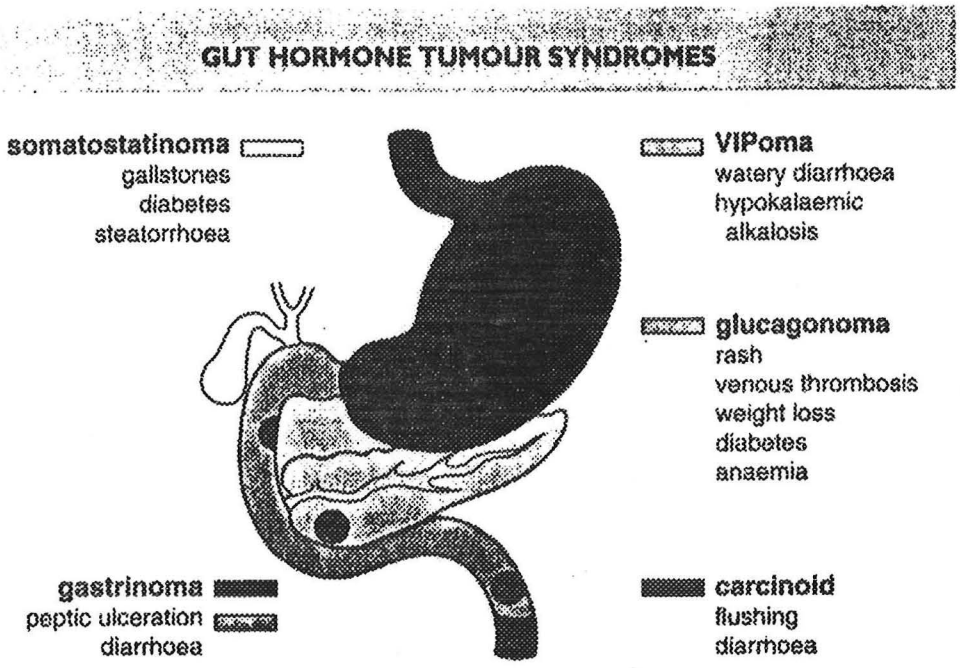


Serum ionized calcium and peripheral midregion parathyroid hormone (PTH) levels after total parathyroidectomy and graft of parathyroid tissue in a patient with MEN 1.

Pancreas. Islet cell tumors of the pancreas are the second most common type of tumor in MEN 1 occurring in 80% of cases [22, 23]. These tumors have a wide range of histology including nesidioblastosis (diffuse hyperplasia throughout the pancreas), microadenoma, macroadenoma, and carcinoma. The tumors are invariably multicentric and more than one histologic type can be present in the same organ [24, 25]. Unlike hyperparathyroidism in which malignancy is rare, metastasis of islet cell tumors in MEN 1 occurs 50% of the time [24].

Islet cell tumors in MEN 1 tend to be pluripotent in their ability to synthesize and secrete hormones. Multiple tumors are often present in the pancreas, not all of which are synthesizing the same hormones. A given tumor is also capable of synthesizing several hormones. Immunhistochemical staining of these tumors often reveals the presence of several different peptides including gastrin, insulin, glucagon, somatostatin, vasoactive intestinal peptide (VIP),

pancreatic polypeptide (PP), neuron-specific enolase, and neurotensin [26]. Despite the variety of peptides produced by these tumors, the endocrine syndromes associated with islet cell tumors in MEN 1 are limited.



Islet cell tumors in MEN 1 are most commonly diagnosed in the fourth to fifth decade [6] but they have been detected from ages 6 to 81 years [27]. Prospective screening of MEN 1 kindreds showed that biochemical abnormalities associated with islet cell tumors can be detected on average at age 25 (range 16-38 years) [28]. In fact, the earliest and most common biochemical abnormalities of MEN 1 were related to islet cell tumors (75% of all patients). Of these, 29% had both pancreatic and parathyroid abnormalities.

The most common islet cell tumor causing an endocrinopathy in MEN 1 is gastrinoma (Zollinger-Ellison syndrome). Historically, the most common cause of death in MEN 1 is GI bleeding from the peptic ulcer disease of Z-E syndrome. Gastrinomas account for 60% of the islet tumors in MEN 1 and are found in approximately 33% of all cases of Zollinger-Ellison syndrome [29]. The clinical features of Z-E are hypergastrinemia causing intractable peptic ulcer disease and diarrhea. As many as 60% of gastrinomas are malignant with 50% of patients having metastatic disease at the time of diagnosis [23]. Surgery (gastrectomy/partial pancreatectomy), H2 blockers, and proton pump blockers are the mainstays of therapy. A detailed discussion of the diagnosis and treatment of Z-E syndrome was recently provided by Dr. McArthur in these Grand Rounds [30].

Insulinoma is the second most common functional islet cell tumor occurring in 35% of MEN 1 cases. Only 4-13% of all insulinomas are associated with MEN 1 [23]. The ratio of benign:malignant insulinomas is 9:1 in sporadic cases, but is 3:1 in MEN 1. The clinical presentation of insulinoma is recurrent hypoglycemia. It can be manifested by neuroglycopenia (altered mental status, syncope, seizure), and increased sympathetic activity (tachycardia, anxiety). Many patients with symptomatic insulinoma eat constantly to maintain a normal blood glucose and thus weight gain is a common feature.

The diagnosis of insulinoma is suggested by the presence of detectable insulin and C-peptide levels during hypoglycemia (glucose <50 mg/dl). Anatomic localization of insulinomas (and most islet cell tumors) is difficult. Ultrasound, CT, and MRI are often not successful in localizing the tumors. Selective pancreatic arteriography and intraoperative ultrasound are the most sensitive imaging techniques currently available. Percutaneous transhepatic portal venous sampling combined with intraarterial calcium injection and intraoperative ultrasound has allowed a surgical cure rate of 92% in patients at the NIH [31].

Comparison of various methods of localizing a single islet-cell adenoma

	Ultrasonography				
	Preoperative	Intraoperative	CT scan	Angiography	THVC
No. of patients	18	18	13	17	7
True positive	61%	89%	46%	70%	71%
False positive	5.5%	5.5%	8%	6%	0%
True negative	0%	0%	0%	0%	0%
False negative	33.5%	5.5%	46%	24%	29%

CT, computed tomography; THVC, transhepatic venous catheterization.

Treatment of insulinoma is surgical with selective resection of pancreatic adenomas or partial pancreatectomy. If the disease is metastatic or surgery has not been successful, diazoxide has been used to reduce insulin secretion and control symptoms [32]. Other medical therapies that have been tried include streptozocin (an islet cell toxin)[33] and dacarbazine [34] as chemotherapeutic agents. Symptomatic relief has been reported with octreotide [35], dilantin [32], propranolol [36], verapamil [37], and glucagon [38].

Plasma glucagon is increased in more than 50% of MEN 1 patients and nearly 30% of islet tumors stain for glucagon. However, glucagonomas are rare and are functionally significant in <5% of MEN 1 pancreatic tumors. These tumors present with hyperglycemia, anemia, venous thrombosis, glossitis, psychiatric disturbances, and a characteristic rash called necrolytic migratory erythema [39]. Most glucagonomas are sporadic. Treatment is surgical and the rash may respond to treatment with octreotide. Other peptides that have rarely been reported to be the

predominant secretory product in MEN 1 include VIP and pancreatic polypeptide. VIPomas present with large volume, watery diarrhea whereas PPomas are clinically silent. Somatostatinoma has not been reported in MEN 1. More than one of these hormones can be secreted simultaneously from the same tumor or from different tumors within the same pancreas, but a clinical syndrome of hormone excess typically is caused by only one hormone (gastrin or insulin).

Pituitary. Pituitary adenomas are found in 50%-65% of MEN 1 patients in autopsy studies but only 16%-42% have detectable pituitary hormone abnormalities [5]. Among all pituitary adenomas in a large Mayo clinic series, only 2.7% were found in MEN 1 patients [40]. Prolactinomas make up more than 75% of the pituitary adenomas in MEN 1 [41] and they are the third most common primary manifestation of MEN 1 (#1 = hyperparathyroidism, #2 = peptic ulcer). Growth hormone secreting adenomas are the second most common pituitary adenoma in MEN 1. Almost all GH adenomas arise primarily within the pituitary but there are rare cases of GH releasing hormone (GHRH) secreting tumors. Corticotrophin (ACTH) and TSH secreting adenomas have been seen in MEN 1, but associated syndromes (Cushing's diseases, TSH-induced thyrotoxicosis) are rare. It is possible that many of these tumors synthesize and secrete immunoreactive hormones that are biologically inactive.

PATIENTS WITH MEN 1 AND PITUITARY TUMORS (MAYO CLINIC SERIES)

1500 consecutive pituitary adenomas 41 (2.7%) associated with MEN 1	
Clinical presentation	
Symptoms related to pituitary tumor	21
Hyperparathyroidism	13
Functional islet cell tumor	2
Size	
Microadenoma (<10 mm)	11
Macroadenoma (≥10 mm)	30
Histochemical profile	
PRL	16
GH/PRL/glycoprotein	7
GH/PRL	6
GH	4
ACTH	3
Null	3
PRL/TSH	1
GH/ACTH/glyocoprotein	1

PRL = prolactin, GH = growth hormone, ACTH = corticotropin, TSH = thryotropin.
Data from Scheithauer BW, Laws ER Jr, Kovacs K, et al: Pituitary adenomas of the multiple endocrine neoplasia type 1 syndrome. Semin Diagn Pathol 4:205–211, 1987; with permission.

PATIENTS WITH MEN 1 AND PITUITARY TUMORS (MD ANDERSON CANCER CENTER SERIES)

11 patients with MEN 1 and concomitant pituitary tumor 6 female, 5 male	
Mean age (yrs) at diagnosis of MEN 1	30
Mean age (yrs) at first pituitary surgery	32
Pituitary endocrine hypersecretion	9
PRL	1
PRL/GH	1
None	
Symptoms of pituitary disease	
Amenorrhea	6
Galactorrhea	4
Visual field defect	4
None	2
Acromegalic features	1
Failure to develop secondary sex characteristics	1
Stigmata of MEN 1	
Hyperparathyroidism	8
Islet-cell tumor	5
Persistent peptic ulceration	2
Multiple pancreatic neoplasms	2
Pancreatic carcinoma	1
Pancreatic mass (undefined)	1
Positive family history without personal history of pancreatic or parathyroid lesion	1

PRL = prolactin, GH = growth hormone.

Many pituitary tumors in MEN 1 are not clinically apparent until the fourth decade. Prolactinomas present with amenorrhea and galactorrhea in women and in men they may be asymptomatic until they attain a large size that by mass effect will produce hypopituitarism and visual field defects from optic chiasm compression. They are easily diagnosed by measurement of serum prolactin and by MRI. Treatment almost always consists of bromocriptine to which these tumors are quite sensitive.

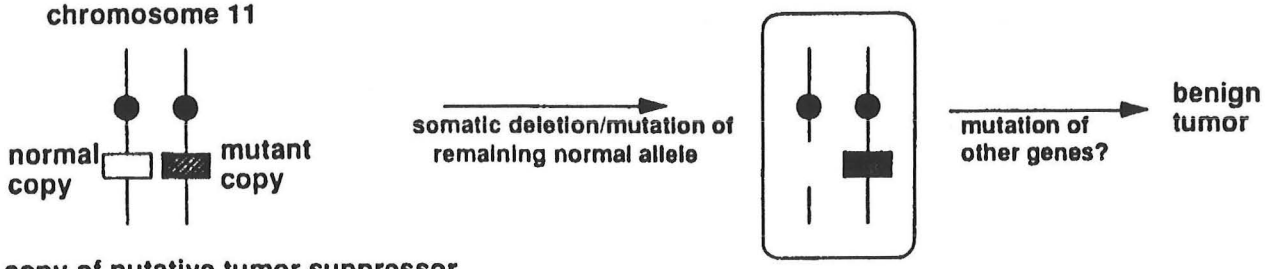
Growth hormone secreting adenomas can rarely present with accelerated growth in children (gigantism). More commonly they present with acromegaly in adults. Diagnosis is made on the basis of serum growth hormone and somatomedin C levels and imaging by MRI. Treatment is surgical, and for recurrent or refractory cases, radiation and/or octreotide can be used.

Other tumors. A variety of other tumors have been reported in various MEN 1 patients. Carcinoid tumors (thymic, bronchial, gastric, and foregut) are the most common of this miscellaneous group. Thyroid carcinomas and lipomas also occur with greater frequency in MEN 1 patients [5].

MEN 1: Genetics

It has long been known that MEN 1 follows an autosomal dominant mechanism of inheritance. It is hypothesized that the MEN 1 gene is a tumor suppressor, and that the development of neoplasia must involve two genetic hits (mutation of one allele and loss of the other normal allele) [42]. Evidence to support this was first reported in 1988 when the MEN 1 locus was identified on chromosome 11 [43]. That study narrowed the locus to the 11q13 region close to the skeletal muscle glycogen phosphorylase gene (PYGM). Analysis of insulinoma tumors from identical twins confirmed that the tumors had lost one allele in 11q13 and that the lost allele was from the unaffected father. The mutated allele was inherited from the affected mother. Thus the two hit hypothesis (inactivation of both alleles) was strongly supported as was the contention that the MEN 1 gene is a tumor suppressor.

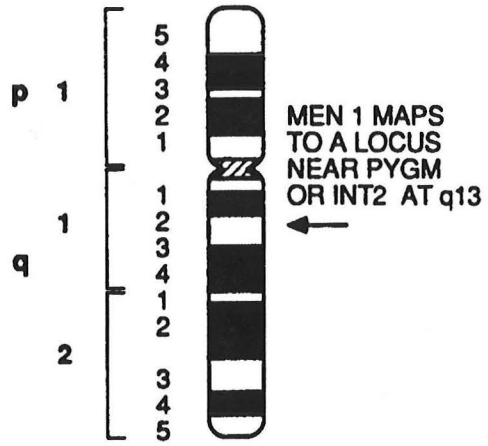
TWO HIT HYPOTHESIS FOR DEVELOPMENT OF MEN 1 TUMORS



Mutant copy of putative tumor suppressor gene on 11q13 is inherited in MEN-1 and present in all parathyroid cells

Clonal progenitor cell lacks functional gene product

Using more precise chromosomal markers and linkage analysis in affected families, the MEN 1 locus has been narrowed down from 12 Mb in 1989 [44] to 1.7 Mb in 1992 [45] to 0.9 Mb in 1994 [46]. A number of candidate genes lie within this region [47] but thus far, none of them have proven to be the MEN 1 gene (see following table).



CANDIDATE GENES FOR MEN 1

Human stomach cancer factor 1 (HSTF1)

B-cell leukemia 1 (BCL1)

Protein phosphatase 1, alpha isoform (PPP1A)

Phospholipase C, beta 3 isoform (PLCß3)

Zinc finger MEN (ZFM1)

HSTF1 is a protein with homology to fibroblast growth factor (FGF), BCL1 is an unknown protein identified as a target for chromosomal break points in B cell leukemias, PPP1A and PLCß3 are enzymes implicated in hormone signalling pathways, and ZFM1 is a putative transcription factor. Given the rate at which the Human Genome Project is proceeding, it is likely that the MEN 1 gene will have been sequenced within the next year if it hasn't been already. Confirmation of its involvement in the disease will likely occur shortly thereafter.

PRIMARY BIOCHEMICAL SCREENING PROGRAM

Parathyroid hormone
Albumin-corrected total serum calcium
Prolactin
Somatomedin C
Blood glucose
Insulin
Proinsulin
Pancreatic polypeptide (PP)
Glucagon
Gastrin
Meal test with PP and gastrin analysis

MEN 1: Screening

Family history can be extremely helpful in identifying patients with MEN 1. However, it is desirable to identify individuals at risk before significant complications arise, and to avoid unnecessary biochemical testing in unaffected family members. One Swedish group reported that RFLP analysis using a combination of 13 different markers (17 DNA probes) near the MEN 1 locus in six different families provided >99.5% predictive accuracy if 3 of the marker systems

were informative [48, 49]. This type of genetic analysis is not widely available, and improved genetic screening must await cloning of the MEN 1 gene.

Biochemical screening is currently used to identify affected individuals in MEN 1 kindreds. The sensitivity of biochemical testing will vary depending on the number of tests used. Using a battery of hormone tests, asymptomatic individuals with MEN 1 can be detected on average at age 18 [28]. However, this approach is not cost effective as it will not likely alter the management of these patients. Biochemical testing for MEN 1 can be divided into two types: screening for carrier status and screening of known carriers (see table below).

BIOCHEMICAL SCREENING FOR MEN 1

	SCREENING FOR CARRIERS		SCREENING KNOWN CARRIERS	
TEST	FREQUENCY	AGE AT TESTING	FREQUENCY	AGE AT TESTING
Serum calcium	q 3-5 yrs	15-50 yrs	q 3-5 yrs	20-50 yrs
Serum prolactin	q 3-5 yrs	>15 yrs	q 3-5 yrs	>15 yrs
Serum gastrin	not indicated*	not indicated	q 3-5 yrs	>25 yrs
Pituitary imaging§	not indicated	not indicated	q 5-10 yrs	20-60 yrs

***Measure serum gastrin in all family members with symptoms of Z-E syndrome at any age.**
§The need for pituitary imaging is controversial if pituitary hormone measurements are normal.

R.F. Gagel
Williams Textbook of Endocrinology, 8th ed.

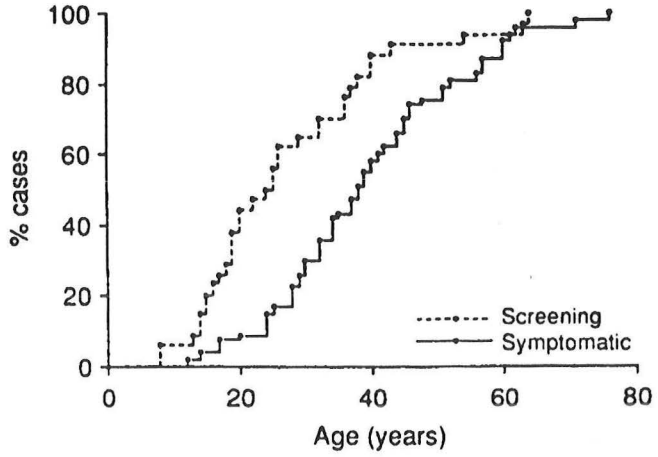


Figure 5. Age-related onset of familial MEN 1. The ages for diagnosis in 87 patients with familial MEN 1 ranged from 8 to 76 years. The patients were subdivided into two groups, depending on the method used to detect MEN 1. The symptomatic group consisted of 53 patients, and the age-related onset for MEN 1 in these members at 20, 35, and 50 years of age was 9%, 43%, and 75%, respectively. In another 34 asymptomatic patients, MEN 1 was detected by biochemical screening, and the respective age-related onset for MEN 1 in these members increased to 44%, 74%, and 91%. Thus, biochemical screening detected an earlier onset of MEN 1 in all age groups.

MULTIPLE ENDOCRINE NEOPLASIA TYPE 2 (MEN 2)

The first description of MEN 2 was by Sipple in 1961 [3]. It is inherited in autosomal dominant fashion with age-related expression. The MEN 2 syndromes occur in two distinct groups, MEN 2A and MEN 2B. MEN 2B has also been termed MEN 3, but with identification of the causative gene, MEN 2B is the designation currently preferred. The similarities and differences of the MEN syndromes are outlined in the following table.

THE MEN 2 SYNDROMES

	MEN 2A	MEN 2B
Medullary thyroid carcinoma	100 %*	100 %
Pheochromocytoma	10-50 %	10-50 %
Hyperparathyroidism	10-20 %	rare
Associated conditions	Hirschsprung's disease Lichen amyloidosis	Mucosal neuromas Intestinal ganglioneuromatosis Marfanoid habitus Thickened corneal nerves Skeletal anomalies Delayed puberty

*Percent of gene carriers who will develop the lesion.

Medullary thyroid carcinoma. Medullary thyroid carcinoma (MTC) makes up 5-10% of all thyroid malignancies (approximately 1500 new cases per year in the U.S.). Most (75-80%) cases of MTC are sporadic. The remaining 25% (300-400 new cases per year) are familial with most cases occurring in MEN 2 and a small number of cases as familial MTC (FMTC). MTC occurs in 100% of patients with MEN 2, usually before the age of 20 (as young as age 2). The earliest lesion is hyperplasia of the parafollicular (C) cells in the thyroid (similar to chief cell hyperplasia in MEN 1). However, unlike the MEN 1 syndromes, the lesion invariably progresses to carcinoma with metastases if left untreated. C-cell hyperplasia is always multifocal and MTC is usually multicentric.

Mortality for all types of MTC is dependent on tumor size and spread. Ten year survival rates for all forms of MTC are >95% for tumors < 1 cm, 50-95% for tumors 1-4 cm, 15-50% when lymph node metastases are present, and <15% when distant metastases are present. MTC causes significant morbidity and mortality in MEN 2 patients. Medullary thyroid carcinoma in MEN 2B is more aggressive, appears earlier (before age 10 [50]), and is the primary cause of

death in 63% of MEN 2B patients [51]. MTC is the primary cause of death in MEN 2A in only 12.5% of cases [51]. FMTC also shows multicentric origins but occurs later in life (>50 years) and is less aggressive than other forms of MTC.

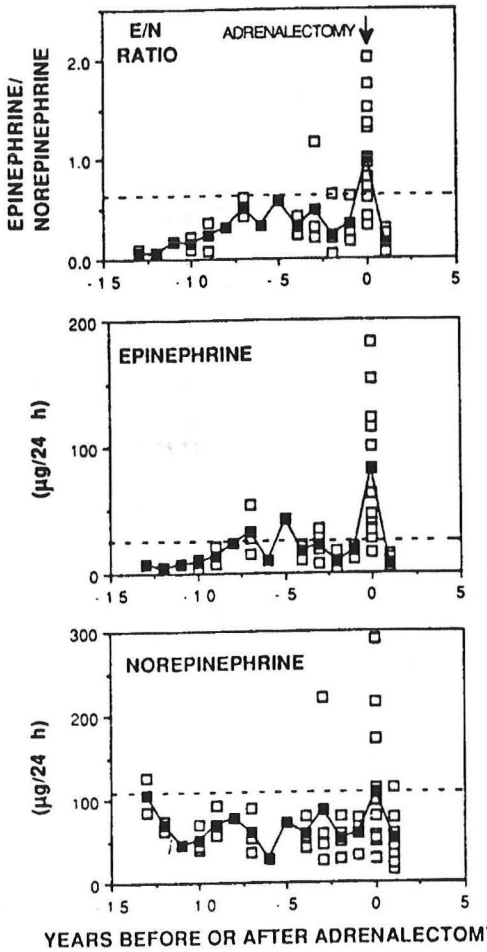
The thyroid C-cells synthesize and secrete calcitonin that can be used as a biochemical marker for disease progression. Several other peptides can also be secreted by MTC and will occasionally produce symptoms. The diagnosis of MTC is usually based on the presence of elevated serum calcitonin levels with or without stimulation by pentagastrin. In known MEN 2 families, all affected individuals will eventually develop C-cell hyperplasia. Until recently, the gene carriers have been diagnosed by routine screening with pentagastrin stimulation tests. This allows for removal of the thyroid before metastatic disease develops. Some cases of MEN 2 will be identified only after MTC is more advanced, and these patients will have detectable thyroid nodules and elevated calcitonin. Fine needle aspiration biopsy of the nodule can be used to diagnose MTC by an experienced cytopathologist. However, the diagnosis might not be made until after surgery.

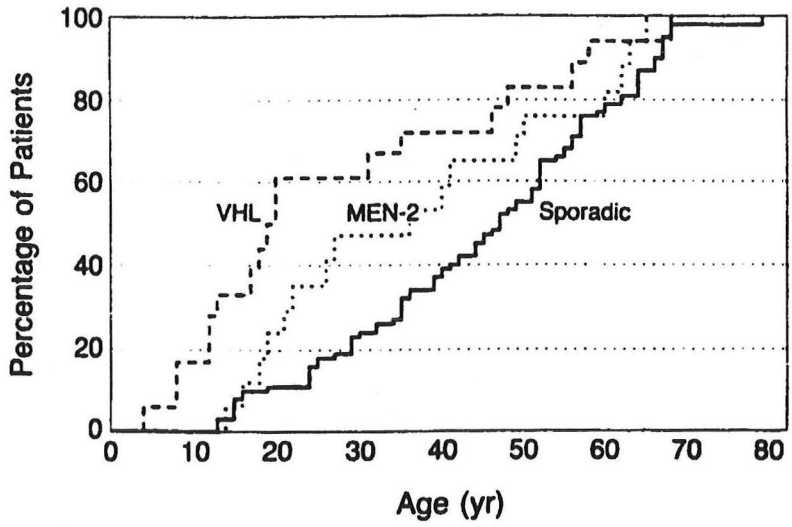
MEDULLARY CARCINOMA

associated clinical syndromes		peptide hormones secreted	
intractable diarrhoea	in 20% of cases	calcitonin	always
		somatostatin	common
		carcinoembryonic antigen	common
		gastrin releasing peptide (bombesin)	common
carcinoid syndrome	rare	histaminase	common
		serotonin	rare
		ACTH	rare
		corticotrophin releasing activity	very rare
		prolactin stimulating activity	very rare
Cushing's syndrome	very rare	nerve growth factor	very rare

Treatment of MTC (sporadic or MEN 2) is total thyroidectomy and central node dissection if distant metastases are absent. All patients should be screened for pheochromocytoma prior to thyroid surgery. Local lymph node metastases can be quite small and easily overlooked. Their presence is often detected post-operatively by persistent elevation of calcitonin levels. If persistent or recurrent disease is present, one group advocates reoperation with extensive neck dissection to eradicate as much occult tumor as possible [52]. This approach has been somewhat successful in reducing serum calcitonin levels but it is not known if this will affect survival rates. When distant metastases are present, total thyroidectomy does not improve survival. There are no effective chemotherapeutic, radiation or hormonal therapeutic options at this time.

Pheochromocytoma. Pheochromocytoma manifests as persistent or episodic hypertension, tachycardia, anxiety, and tremor. The symptoms result from excessive secretion of catecholamines from hyperplastic or adenomatous adrenal medullary chromaffin cells. Pheochromocytomas develop in 10-50% of MEN 2 patients [53], and are usually detected later in life (mean age 36 years [51]). The later detection probably reflects the less sensitive screening methods for pheochromocytoma compared to MTC. Among MEN 2-related pheochromocytomas, 90% are found in MEN 2A and 10% are MEN 2B. The pheochromocytomas in MEN 2 are bilateral 70-80% of the time reflecting the multicentric involvement characteristic for these syndromes [51]. Approximately 4% of pheochromocytomas are malignant in MEN 2. Somewhat surprising is that pheochromocytoma was the primary cause of death in 74% of patients with MEN 2A and 25% in MEN 2B [51].





Age of the Patients at the Time of Diagnosis of Symptomatic Pheochromocytoma.
The cumulative age distributions of 43 patients with von Hippel–Lindau disease (VHL), 24 patients with MEN-2, and 63 patients with sporadic pheochromocytoma are shown.

The diagnosis of pheochromocytoma can be challenging if the tumor functions episodically as it often does in sporadic cases. In MEN 2, the multicentric hyperplasia results in more persistent catecholamine secretion. Consequently, routine screening with 12 or 24 hour urine testing should be adequate to identify most pheochromocytomas in MEN patients. The most sensitive tests remain the 24 hour urine for metanephrine, vanillylmandelic acid (VMA),

and catecholamines. Positive urine screening should be followed by abdominal CT. If a mass cannot be visualized and extra-adrenal pheochromocytoma is suspected, imaging with 131-I MIBG can be helpful in localizing the tumor.

Treatment is surgical with unilateral adrenalectomy initially in most cases. The contralateral gland is explored and if it appears abnormal it is also removed. Approximately 50% of the remaining adrenal glands will develop pheochromocytomas at 10 years after the first surgery. As with many neuroendocrine tumors there are no effective non-surgical therapies for malignant pheochromocytomas.

Pheo in MEN 2: comparison between 2A (274) and 2B (26 patients)

	MEN 2	
	2A	2B
Total (%)	91.3	8.7
Mean age at pheo (years)	39.5	32.4
Involvement (%):		
unilateral	33.3	21.1
bilateral	66.7	78.9
Chronology (%)		
pheo first	27.6	4.2*
simultaneous	32.1	33.3
pheo second	40.3	62.5*
Death (%)		
MTC	12.9	62.5**
pheo	74.2	25
other causes	12.9	12.5

* $P < 0.05$.
** $P < 0.01$.

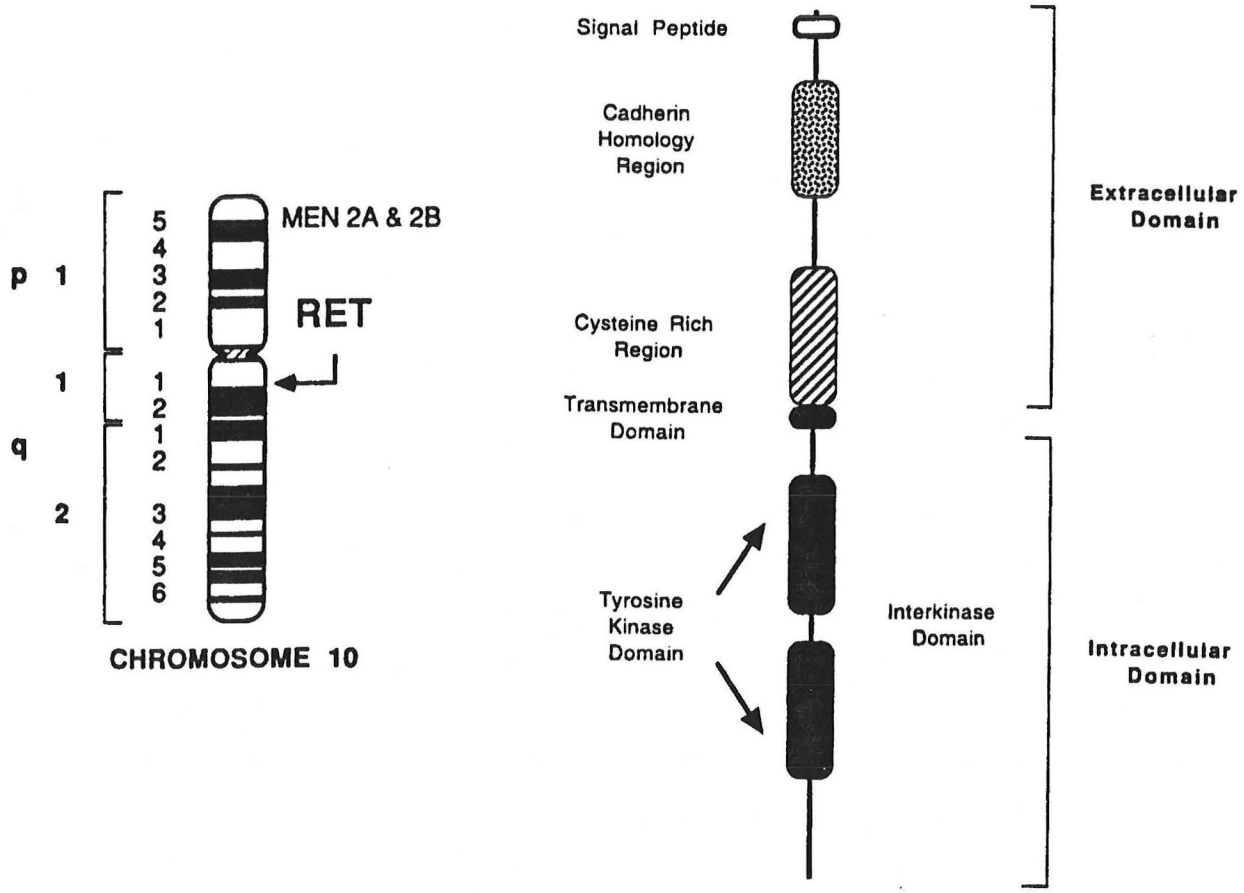
Hyperparathyroidism. The hyperparathyroidism of MEN 2 is similar to MEN 1 in that the earliest lesion is diffuse chief cell hyperplasia [54]. However, the hyperparathyroidism of MEN 2 occurs later in life (third decade), is less likely to present with hypercalcemia, and is less prevalent (10-50%)[55]. Some MEN 2 families rarely have hyperparathyroidism while others may have as many as 25% affected members [56]. Prospective screening for MTC often results in early thyroidectomy and partial parathyroidectomy which may account for the lower incidence of hyperparathyroidism in MEN 2. The diagnosis and treatment of hyperparathyroidism in MEN 2 is the same as in MEN 1.

Associated conditions. The MEN 2 syndromes are occasionally associated with other clinical findings. MEN 2A has been reported in three families with hereditary cutaneous lichen amyloidosis (CLA) [57-59]. Like MEN 2, hereditary CLA has an autosomal dominant mode of transmission.

MEN 2B is characterized by several abnormalities. Neuromas of the eyelids, tongue, lips, and oral mucosa develop after the age of 2 years. Ganglioneuromas develop in the pancreas, gallbladder, salivary glands, and along the alimentary tract. Thickening of the corneal nerves is observed on slit lamp exam [60], and a marfanoid habitus with skeletal abnormalities is frequently seen. Most of these conditions are apparent in childhood.

MEN 2: Genetics

The MEN 2 locus was localized in 1987 near the centromere of chromosome 10 [61, 62]. In 1993, the locus was narrowed to a 480 kb region [63, 64] in which the RET protooncogene was eventually identified as the MEN 2 gene [65, 66]. Molecular characterization of MEN 2 has proceeded rapidly since then.



Schematic diagram of the RET tyrosine kinase receptor.

RET protooncogene. The RET (*RE*arranged during *T*ransfection) protooncogene was first identified by its ability to transform NIH-3T3 fibroblasts that had been transfected with tumor DNA [67]. It was subsequently cloned and found to be a member of the protein tyrosine kinase family of oncogenes [68]. This gene family includes the receptors for epidermal growth factor (EGF), colony stimulating factor-1, and insulin-like growth factor 1. Related members include the protooncogenes met, neu, and trk. RET is most closely related to the EGF receptor. The RET gene spans 80 kb, contains 21 exons, produces five major mRNA transcripts (7.0, 6.0, 4.6, 4.5, and 3.9 kb) and encodes two protein isoforms of 1072 and 1114 amino acids that differ at their carboxy termini [69]. Its amino acid sequence predicts a transmembrane protein with an extracellular amino terminus (exons 1-10) containing a cysteine-rich domain, and a region with homology to cadherins (calcium-dependent extracellular adhesion proteins). The intracellular carboxy terminus contains the tyrosine kinase domain. It is assumed that this protein is a receptor for a yet to be identified ligand.

The RET gene is expressed in cells of the neural crest as early as embryonic day 8.5 in the mouse [70]. It can be found in brain, autonomic and enteric ganglia, thyroid C-cells, adrenal medulla, parathyroid gland, Wolffian duct, and ureteric bud. It is expressed in high levels in neuroblastomas, MTC, and pheochromocytoma [71]. Its expression is low or absent in most adult tissues. Deletion of RET in mice results in absence of enteric ganglia and renal agenesis [72]. These data suggest that the normal function of RET is in the migration, proliferation, and differentiation of neural crest cells and the kidney.

RET mutations in MEN 2. Cloning of the MEN 2 gene provided a tool for genetic diagnosis that is now being used widely. Direct sequencing of the RET protooncogene using genomic DNA has identified several mutations that are distributed over the entire length of the gene (see table). The majority of mutations in MEN 2A are found clustered at cysteine residues in exon 10 (codons 609, 611, 618, and 620) and exon 11 (codon 634). All of these are missense point mutations that result in substitution of the cysteine by another amino acid. More than 87% of the MEN 2A RET mutations are in codon 634 [73]. Mutations in FMTC are found with approximately equal frequency in codons 618, 620, and 634.

A striking feature of MEN 2B is that 100% of cases with an identifiable mutation are due to a single point mutation in codon 918 (ATG -> ACG) converting a Met to a Thr residue [73]. When 25 affected individuals were studied, it was determined that in all cases, the mutated RET allele was of paternal origin, and that there appeared to be a paternal age effect [74]. Thus, MEN 2B can be added to the list of neoplastic syndromes in which the genetic alteration is predominantly or exclusively paternal (Wilms tumor, bilateral retinoblastoma, osteosarcoma, embryonal rhabdomyosarcoma, and neurofibromatosis type I). The codon 918 mutation (non-

germline) has been detected in at least 33 cases of sporadic MTC [75], and in several sporadic pheochromocytomas [76, 77].

RET GERMLINE MUTATIONS[a]

Germline RET mutations (codon; nucleotide or gross change)	*Exon*	*Phenotype and reported cases (number)*[b]
32TCG → TTG	2	HSCR alone (1)[67]
64CCC → CTC	2	HSCR alone (1)[67]
136GAG → TAG (stop)	3	HSCR alone (1)[67]
180CGA → TGA (stop)	3	HSCR alone (1)[67]
330CGG → CAG	5	HSCR alone (1)[67]
del G after 373GCG (frameshift)	6	HSCR alone (1)[68]
393TTC → TTA	6	HSCR alone (1)[67]
609TGC → TAC	10	FMTC (1)[44]
609TGC → TGG	10	HSCR alone (1)[73]
611TGC → TGG	10	MEN 2A (1)[92]
618TGC → CGC	10	MEN 2A (2)[20,92]; FMTC (2)[58,93]; MEN 2A/HSCR (1)[73]
618TGC → GGC	10	MEN 2A (1)[21]
618TGC → AGC	10	MEN 2A (3)[20,56,92]; FMTC (3)[20,56]; o.h. MTC (1)[56]
618TGC → TAC	10	MEN 2A (1)[92] FMTC (1)[56]
618TGC → TCC	10	FMTC (3)[93]
618TGC → TTC	10	MEN 2A (1)[92]
620TGC → CGC	10	MEN 2A (6)[20,56,58,92,93]; o.h. MTC (4)[56]; MEN 2A/HSCR (2)[73]; FMTC/HSCR (1)[73]
620TGC → TAC	10	MEN 2A (2)[20,92]
620TGC → TCC	10	MEN 2A (1)[56]; FMTC (1)[56]; o.h. MTC (1)[56]
620TGC → TTC	10	MEN 2A (3)[56,92,93]; FMTC (1)[56]
634TGC → CGC	11	MEN 2A (72)[44,56,58,88,90,92,93]; FMTC (2)[58]; o.h. MTC (1)[56] MEN 2A/CLA (1)[56]
634TGC → GGC	11	MEN 2A (8)[44,56,58,88,92]
634TGC → AGC	11	MEN 2A (4)[44,56,58,92]; o.h. MTC (1)[56]
634TGC → TAC	11	MEN 2A (31)[44,56,58,88,92,93]; FMTC (2)[44]; o.h. MTC (1)[56]; MEN 2A/CLA (1)[85]
634TGC → TCC	11	MEN 2A (5)[44,56,58]; FMTC (1)[56]
634TGC → TTC	11	MEN 2A (6)[44,56]; FMTC (2)[49,93]; MEN 2A/CLA (1)[56]
634TGC → TGG	11	MEN 2A (7)[44,56,90,92]; o.h. MTC (1)[56]
765TCC → CCC	13	HSCR(sp) alone (1)[68]
768GAG → GAC	13	FMTC (1)[45]
897CGA → CAA	15	HSCR(sp) alone (1)[68]
918ATG → ACG	16	MEN 2B (72)[22–24,88]
972AGG → GGG	17	HSCR alone (1)[68]
del 10q11.2–21.2 (*RET* deleted)	N/A	HSCR(sp) alone (1)[68]
Microdeletion in 10q11.2 (*RET* deleted)	N/A	HSCR alone (1)[68]

[a]HSCR, familial Hirschsprung's disease; HSCR(sp), sporadic Hirschsprung's disease; FMTC, familial medullary thyroid carcinoma; MEN 2A, multiple endocrine neoplasia type 2A; MEN 2B, multiple endocrine neoplasia type 2B; CLA, cutaneous lichen amyloidosis and o.h. MTC, other hereditary medullary thyroid carcinoma (56). Mutated nucleotides are underlined. Families reported in more than one publication have been counted once only and attributed to the most recent publication.

[b]Superscript numbers are reference numbers.

RET SOMATIC MUTATIONS[a]

Ret somatic mutations (codon; nucleotide change)	*Exon*	*Cases identified (number)*[b]
768GAG → GAC	13	(4)[45]
883GCT → TTT	15	(2)[51]
918ATG → ACG	16	(33)[24,48,49,50]

[a]Mutated nucleotides are underlined.

[b]Superscript numbers are reference numbers.

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MUTATIONS IN THE RET PROTOONCOGENE
International RET Mutation Consortium

	Number of families	Percent of total cases	RET mutation positive	RET mutation negative
MEN 2A	147	62.8 %	95.2 %	4.8 %
MEN 2B	64	27.4 %	93.8 %	6.2 %
FMTC	23	9.8 %	87.0 %	13.0 %
TOTAL	234	100 %	94.0 %	6.0 %

	MUTATED CODON (PER CENT TOTAL MUTATIONS)					
	609	611	618	620	634	918
MEN 2A	0.7	2.1	3.5	6.4	87.1	0
MEN 2B	0	0	0	0	0	100
FMTC	5.0	0	35.0	25.0	30.0	0

Adapted from J. Intern. Med. 238:343 (1995)

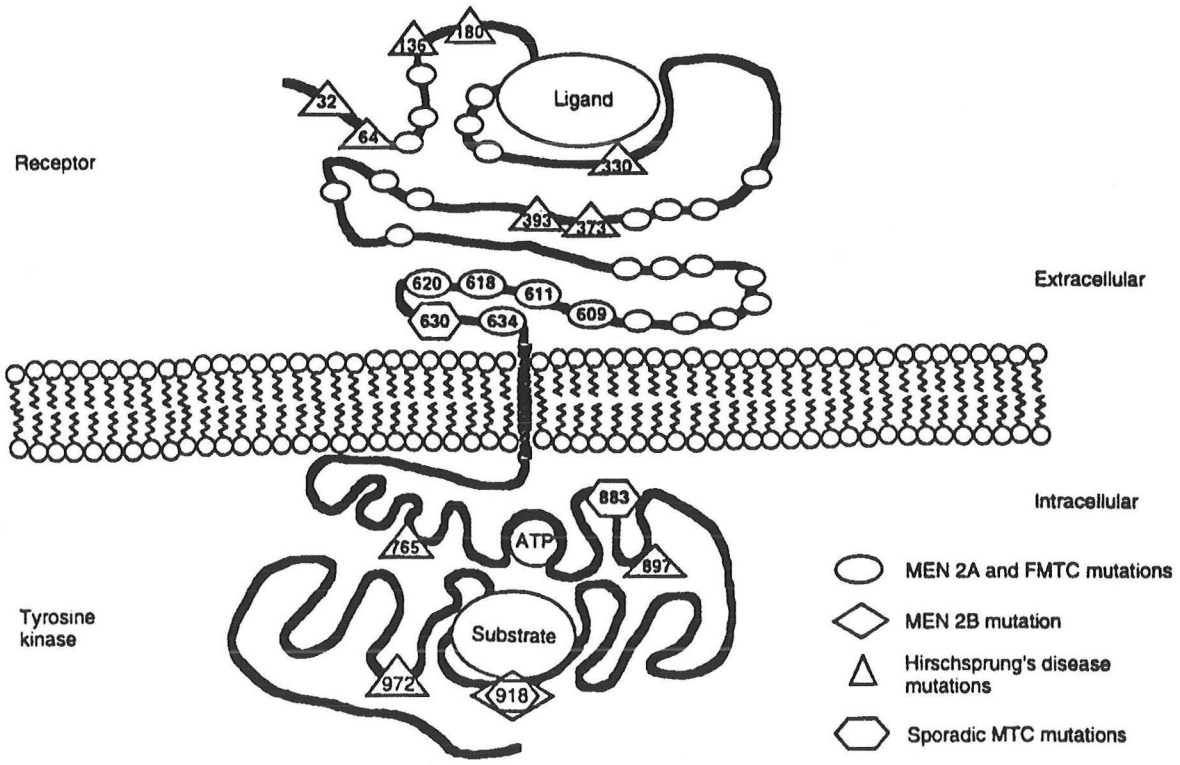


Fig. 1 Graphic representation of the RET proto-oncogene protein domains and mutations associated with disease: MEN 2A/2B/FMTC/Sporadic MTC/Hirschsprung's mutations in the RET proto-oncogene. All reported mutations in the RET proto-oncogene that result in MEN 2A and FMTC are indicated (codons 609, 611, 618, 620 and 634) [12, 16, 5, 17]. Three RET mutations that are associated with sporadic cases of MTC are also indicated (codons 630, 883 and 918) [12, 14, S. Dou and H. Donis-Keller, unpublished results]. The mutation at codon 918 is shown in its position at the catalytic core region of the tyrosine kinase domain [13, 14, 15]. Reported mutations that are associated with Hirschsprung's disease are also shown (codons 32, 64, 136, 180, 330, 373, 393, 769, 8883, 897 and 972) [18, 19].

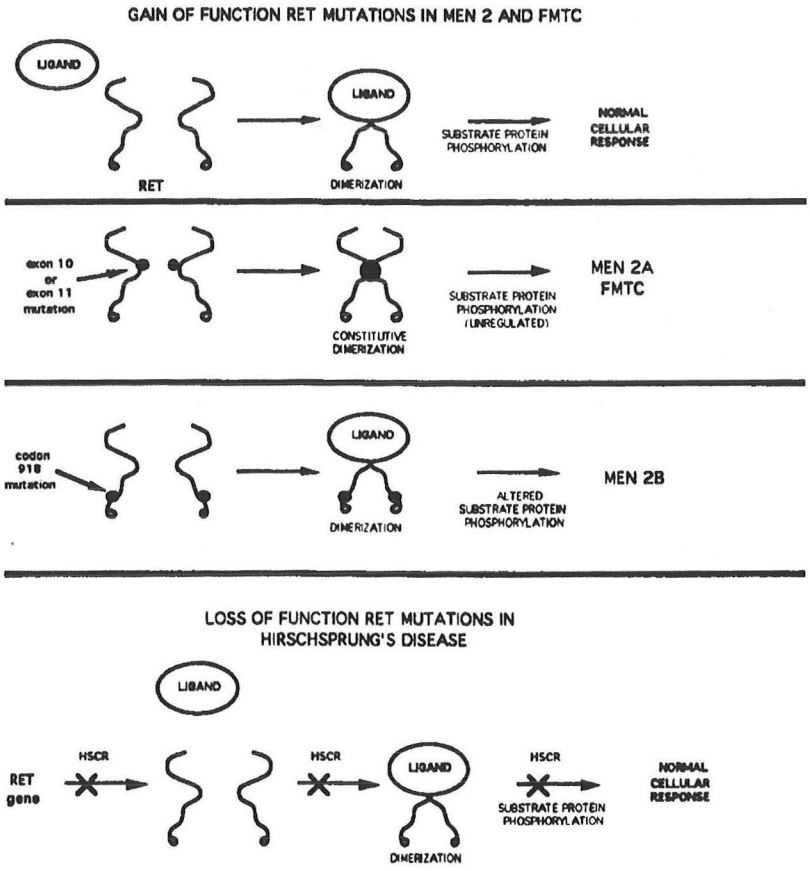
H. Donis-Keller
J Intern Med. 238: 319 (1995)

RET and Hirschsprung's disease. Hirschsprung's disease (HSCR, aganglionic megacolon) is a disease with heterogeneous genetic causes. One autosomal dominant form of HSCR was localized to chromosome 10q11.1 (i.e. near the RET locus) [78]. Analysis of patients in selected families with this form of HSCR showed germline RET mutations as the probable cause for HSCR [79, 80]. The RET mutations responsible for HSCR are scattered over the length of the RET gene and do not coincide with those that cause MEN 2 [81]. MEN and HSCR have not been found in the same patients or in the same families. Mutations in RET may be responsible for only 10% of cases of HSCR indicating that HSCR is a heterogeneous genetic disorder with more than one gene involved. This fact is highlighted by a study (involving Dr. Yanagisawa of the Howard Hughes Institute at UT Southwestern) showing that one recessive form of HSCR in humans is caused by mutation in the endothelin-B receptor [82].

RET and papillary thyroid carcinoma. Prior to its identification as the MEN 2 gene, the RET protooncogene was found to be involved in the development of papillary thyroid carcinoma. Somatic mutations were found in 25% of papillary thyroid cancers that contained the carboxyl portion of RET fused to another sequence (papillary thyroid cancer or PTC) to form a chimeric molecule (RET/PTC1) [83, 84]. Subsequent studies have found two other similar rearrangements to form RET chimeras (RET/PTC2, RET/PTC3) in papillary thyroid cancers [85-89]. All of these are somatic mutations, and thus RET rearrangement is implicated in the genesis of at least one other malignancy.

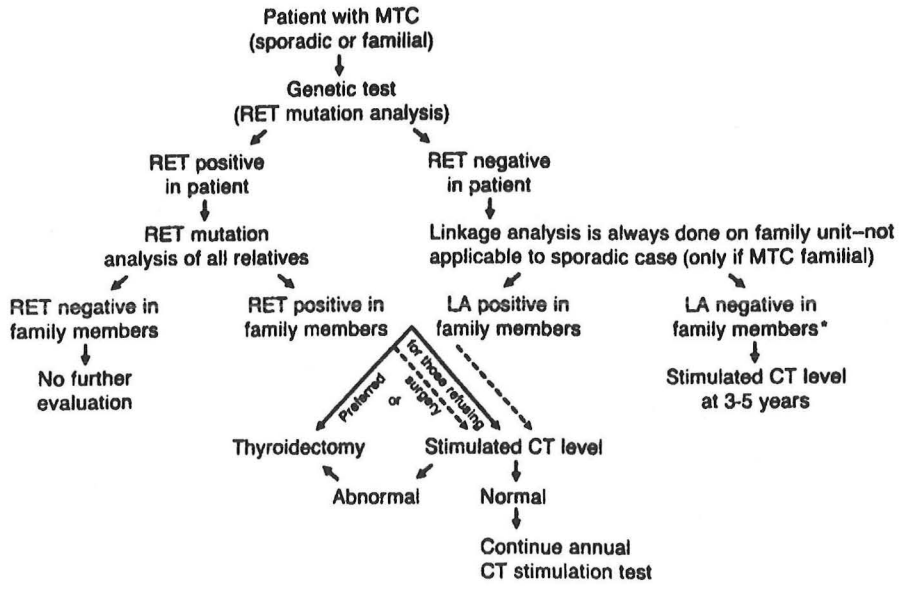
Mechanisms of RET action. How do mutations in the RET gene cause such diverse clinical syndromes as a dominantly transmitted disease [90]? It is predicted that protein tyrosine kinase receptors such as RET normally function by binding to a ligand that causes receptor dimerization and activation of the tyrosine kinase. Most of the MEN 2A mutations occur in the cysteine rich extracellular domain that is thought to be important for dimerization. The codon 634 mutation appears to promote ligand-independent dimerization of RET leading to constitutive activation of the kinase [91, 92]. The codon 918 mutation that causes MEN 2B lies within the intracellular kinase domain, and this mutation alters the catalytic properties of the kinase [91]. The altered catalytic properties of the kinase produce cell transformation possibly by altering the protein substrates that are phosphorylated by RET. Therefore, the codon 918 RET mutation produces a gain of function. The distinct biochemical effects (constitutive dimerization, altered kinase properties) lead to distinct clinical syndromes (MEN 2A, MEN 2B).

RET mutations in HSCR are more heterogeneous and widely scattered over the molecule. Some of them prevent synthesis of a full length protein. It is hypothesized that these mutations lead to inactivation of the RET gene (loss of function). This hypothesis is supported by studies in the RET knockout mouse in which the HSCR phenotype is present [72].



MEN 2: Screening

Identification of RET as the MEN 2 gene has significantly altered the way in which screening for the disease is performed. One proposed algorithm for screening for RET mutations is shown below.



Algorithm suggesting sequence of tests in a patient with medullary thyroid carcinoma (MTC), family screen, and management based on results of genetic testing. Thyroidectomy is recommended for all persons predicted to be gene carriers by either direct DNA mutation analysis or linkage-based testing. Dashed line represents an alternative, less desirable course, when surgical decision is based on follow-up evaluation with pentagastrin tests. *Indicates further evaluation dictated by adequacy of markers to exclude gene carriers. CT = calcitonin; LA = linkage analysis.

RET mutations have not yet been identified in 6% of the cases of MEN 2 and FMTC [73]. Most screening programs look for mutations in exons 10, 11, and 16. It is possible that the RET mutation-negative families will have unique mutations in other exons or in non-coding regions of the RET gene that affect its expression. It is also possible that there may be a small subset of MEN 2 and FMTC families in which the mutation is in another gene that affects RET (e.g. its ligand).

There are several different methods for RET mutation screening all of which depend upon PCR. A rapid screening technique is single strand conformation polymorphism that can detect point mutations [93]. This technique is currently used at UT Southwestern in the Laboratory for Molecular Diagnostics to screen exons 10 and 11. A variation of this technique uses restriction enzyme polymorphism to identify mutations. All putative mutations are verified by direct DNA sequencing of genomic DNA to eliminate false positives and normal polymorphisms [94]. At least one company (OncorMed, Gaithersburg, MD) provides direct DNA sequencing of exons 10, 11, and 16 as the initial test (cost = $420). Many centers require informed consent for testing and offer genetic counseling.

A number of centers currently perform prophylactic thyroidectomy (usually in children) in known carriers of RET mutations [50, 56]. This approach has been shown to be successful in preventing the morbidity of MTC in such patients [56]. An international consortium has recommended prophylactic thyroidectomy after age 6 for all gene carriers [73].

SCREENING FOR MEN 2 AND FMTC

	SCREENING FOR CARRIERS		SCREENING KNOWN CARRIERS	
TEST	FREQUENCY	AGE AT TESTING	FREQUENCY	AGE AT TESTING
RET mutations	once	< 6 years	NA	NA
Pentagastrin stimulation	NA	NA	q 1-2 yrs for 5 yrs then q 5 yrs	lifetime
Urine catecholamines	NA	NA	Yearly	5-60 yrs
Serum calcium*	NA	NA	Biyearly	lifetime

NA = not applicable
*Serum calcium screening is not indicated for MEN 2B.

Adapted from R.F. Gagel
Williams Textbook of Endocrinology, 8th ed.

Lifetime screening costs for multiple endocrine neoplasia type 2A

Group	Pentagastrin/ catecholamines/ serum CA**/patient*	Genetic testing cost/patient
RET mutation	US$5,089†	US$500 + 1,990† = US$2,490
Family member at risk with normal RET proto-oncogene	US$15,587‡	US$500

*Based on test price of $500 for a pentagastrin test, $81.00 for urine catecholamines, $18.50 for a serum calcium measurement and $500 for mutational analysis of the RET proto-oncogene.
†Individuals with RET mutations or detection of MTC by pentagastrin testing (average age of 10 years) will have continued screening for parathyroid and adrenal medullary disease (average age of 25 years).
‡Cost of pentagastrin test, serum calcium, and urine catecholamines from age 4 until age 30 years.

OVERLAP SYNDROMES

Isolated families have been identified that have features of both MEN 1 and MEN 2 [95-99]. The small numbers of affected individuals has not allowed a clear cut mode of transmission to be identified. To my knowledge, the RET gene has not been characterized in these families. It is not known if these represent multigenic syndromes or new gene mutations.

A possible new type of multiple endocrine neoplasia

Age/ sex	Sites of extraadrenal paragangliomas	Pituitary tumor	Parathyroid involvement	Skin findings	Other findings	Family history
36F	Cervical chemodectomy, probably of vagus nerve	Acromegaly	Primary hyperplasia	Not mentioned	Antral and duodenal G-cell hyperplasia; bronchial carcinoid	Not given
19F	Multiple sites	Acromegaly	Primary hyperplasia	Multiple pigmented nevi	None	Father and sister with multiple pigmented nevi. Evaluation refused
61F	Bilateral carotid bodies	Adenoma (unknown function)	Hyperplasia (serum calcium not measured)	Not mentioned	Gastric leiomyoma, papillary thyroid carcinoma	Daughter and granddaughter both normocalcemic with enlarged sella turcica, visual field cuts, and sonographic evidence of bilateral carotid body tumors; intervention refused

GOALS OF FUTURE RESEARCH

Clearly there has been tremendous progress in understanding these syndromes (particularly MEN 2) over the last 2 years. However, there is much to be learned. Research priorities in this field include:

1. Clone the MEN 1 gene.
2. Identify the RET ligand.
3. Develop better methods for imaging islet cell tumors.
4. Develop more sensitive screening methods for early detection of pheochromocytoma.
5. Develop better non-surgical methods for treatment of MEN tumors.

CONCLUSIONS

The MEN syndromes are well-recognized genetic syndromes. Cloning of the MEN 2 gene (RET) has provided significant insight not only into MEN 2 but also (somewhat surprisingly) into Hirschsprung's disease as well. Screening for gene carriers is now readily available and has changed the way in which these patients are managed. Identity of RET as a putative receptor also provides a target for future pharmacologic therapy. It is likely that cloning of the MEN 1 gene will provide similar surprises, insights, and therapeutic changes.

ACKNOWLEDGMENTS

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APPENDIX

Multiple Endocrine Neoplasia 2A and Familial Medullary Thyroid Carcinoma Mutation Analysis (Ret Proto-oncogene Exons 10 and 11)

University of Texas Southwestern Medical Center Laboratory for Molecular Diagnostics
5323 Harry Hines Blvd.
Dallas, TX 75235-9072

Phone: 214-648-4075

Performed: Monday

Methodology: Polymerase Chain Reaction and Single Strand Conformation Polymorphism

Specimen Requirements: Peripheral blood: 10-20 ml EDTA whole blood. Send specimen at room temperature.

Tissue: 5x5x5 mm minimum or 500 mg of tissue. Specimen must be frozen within 1 hour of collection. Send specimen frozen and on dry ice.

Special Instructions: Sample must arrive within 24 hours of draw. Send specimen Monday through Thursday only.

Please include a family pedigree with sample.
Please complete a "Laboratory for Molecular Diagnostics Request Form" and forward it with the specimen.

Rejection Criteria: Clotted peripheral blood. Thawed tissue sample.

Reference Range: The laboratory will provide an interpretive report.

Cost: $175.00 per specimen analyzed