Multiple endocrine neoplasia type 1 (MEN1) consists of benign, and sometimes malignant, tumors (often multiple in a tissue) of the parathyroids, enteropancreatic neuroendocrine system, anterior pituitary, and other tissues. Skin angiofibromas and skin collagenomas are common. Typically, MEN1 tumors begin two decades earlier than sporadic tumors. Because of tumor multiplicity and the tendency for postoperative tumor recurrence, specialized methods have been developed for preoperative and intraoperative localization of many MEN1-associated tumors.

The MEN1 gene was recently isolated by positional cloning. This strategy progressively narrows the size of the candidate MEN1 gene interval on the chromosome and then finds and tests many or, if needed, all genes within that interval. The MEN1 gene was finally identified because it was the one gene that contained mutations in most DNAs from a test panel of MEN1 cases.

It has been suggested that MEN1, like many hereditary cancer syndromes, is caused by mutation in a tumor suppressor gene that contributes to neoplasia when both gene copies in a tumor precursor cell have been sequentially inactivated ("two-hit" oncogenesis mechanism). Germline MEN1 mutations were found in most families with MEN1 and in most cases of sporadic MEN1. In addition, the MEN1 gene was the gene most likely to show acquired mutation in several sporadic or nonhereditary tumors—parathyroid adenomas, gastrinomas, insulinomas, and bronchial carcinoids. Most germline or acquired MEN1 mutations predicted truncation (and thus likely inactivation) of the encoded protein, supporting expectations for the "first hit" to a tumor suppressor gene. Testing for MEN1 germline mutation is possible in a research setting. Candidates for MEN1 mutation testing include patients with MEN1 or its phenocopies and first-degree relatives of persons with MEN1.

Endocrine Neoplasias in Relation to Oncogenes and Tumor Suppressor Genes

Dr. Allen M. Spiegel (Metabolic Diseases Branch, National Institute of Diabetes and Digestive and Kidney Diseases [NIDDK], National Institutes of Health [NIH], Bethesda, MD): Multiple endocrine neoplasia type 1 (MEN1) is an autosomal dominantly inherited disease characterized by variable penetrance for tumors of the parathyroids, enteropancreatic neuroendocrine system, and anterior pituitary and, less commonly, by tumors in other tissues (1). The "multiple" designation refers both to the occurrence of multiple tumors in the involved endocrine organ (for example, multiple pancreatic islet tumors) and to the occurrence of tumors in multiple endocrine organs (for example, parathyroid tumor plus pancreatic islet tumor). In contrast, sporadic endocrine tumors generally occur as a single lesion. Clinical manifestations of MEN1 include functional effects of hormone hypersecretion, such as hypercalcemia or hypoglycemia; mass effects secondary to tumor growth (particularly in the pituitary); and malignant neoplasm (particularly with malignant gastrinoma).

Type 1 disease must be distinguished from type 2 disease (MEN2), another autosomal dominantly inherited tumor syndrome. The latter is characterized by bilateral medullary carcinoma of the thyroid, pheochromocytomas (often bilateral), and, in the most common variant, parathyroid tumors (2).

History

The coexistence of tumors of the parathyroids, pancreatic islets, and pituitary was first noted in autopsy studies of patients with acromegaly (3). In 1939, Rossier and Dressier (4) described two sisters with nephrolithiasis in addition to parathyroid and pancreatic islet tumors. A paternal family history of ulcers was also noted, but the connection to the sisters' endocrine tumors was not made until 22 years later (5). Not until 1954, when Wermer (6) described a family with four daughters and a father manifesting multiple parathyroid, pancreatic islet,
and pituitary tumors, was the autosomal dominant genetic basis of the disease recognized. This condition was later termed the Wermer syndrome or multiple endocrine adenomatosis, but MEN1 is now the preferred term.

Oncogenes and Tumor Suppressor Genes

Two categories of genes contribute to tumorigenesis. First, oncogenes, such as \( RAS, MYC, \) and \( RET, \) normally act to stimulate cell proliferation but when inappropriately activated or overexpressed permit a cell growth advantage. Second, tumor suppressor genes, such as \( P53 \) (mutation of which causes the Li–Fraumeni syndrome) or \( FAP \) (mutation of which causes familial adenomatous polyposis of the colon), normally act as “brakes” on cell proliferation and, when inactivated, permit a cell growth advantage. Knudson (7) studied retinoblastoma and recognized that tumors in familial cases could be explained by an inherited germline loss of function mutation (“first hit”) of a tumor suppressor gene (termed \( RBI \) for retinoblastoma gene) in all cells (Figure 1). This could be followed by a somatic loss-of-function mutation of the second, normal, \( RBI \) allele in one cell (“second hit”). The cell would then be freed from restraint by both brakes (two normal \( RBI \) alleles) and could proliferate into a clonal neoplasm (Figure 1). The presence of the first hit in all cells through germline inheritance and the relative frequency of second hits in many somatic cells may explain the multiple and bilateral retinoblastomas and the early age at tumor onset that are characteristic of the hereditary disease.

Clinical Correlates of Two-Hit Tumorigenesis

Knudson (7) suggested that sporadic retinoblastoma, in contrast to familial retinoblastoma, could arise after two sequential inactivating somatic mutations of the \( RBI \) gene occur in a single cell (Figure 1). The lower frequency with which these two events occur postnatally in a single cell is reflected in the unilateral, single nature of the tumor and the later age at tumor onset in the sporadic form of the disease.

Although a tumor suppressor gene is recessive at the tumor level (both alleles in a cell must be inactivated for the cell to proliferate as a tumor clone), a heterozygous germline mutation combined with the frequent occurrence of somatic, second hits leads to dominant inheritance and dominant expression of tumors. A tumor suppressor gene can contribute to benign or malignant neoplasia and can synergize with other tumor suppressor genes or oncogenes.

Second Hits and the Role of Multiple Endocrine Neoplasia Type 1 in Hereditary and Sporadic Tumors

Second hits are often large (for example, loss of an entire copy of a chromosome), and this DNA change is also present in the monoclonal cellular progeny that grow from this tumor precursor cell. Thus, indirect evidence for a large second hit is often found when one copy of a heterozygous DNA marker on either side of a tumor suppressor gene is lost in tumor DNA compared with constitutional DNA. This is termed loss of heterozygosity or allelic loss.

In 1988, pedigree testing first linked the \( MEN1 \) gene to a section of chromosome 11, \( 11q13 \) (8). An analysis of loss of heterozygosity of polymorphic markers that were mapped near this locus in islet-cell tumors from brothers with MEN1 showed that the presumed normal allele, inherited from the unaffected parent, had been lost (8). Similar observations were subsequently made for parathyroid tumors from patients with MEN1 (9, 10). Furthermore, loss of heterozygosity at \( 11q13 \) was also seen in about one third of sporadic parathyroid adenomas (9). These observations suggested that 1) \( MEN1 \) is a tumor suppressor gene; 2) MEN1 is a familial tumor syndrome caused by a germline inactivating mutation of the \( MEN1 \) gene, with frequent somatic second hits leading to multiple endocrine tumors; 3) the same gene has a similar role in certain sporadic tumors; and 4) a MEN1-related tumor is a monoclonal or oligoclonal expansion after the second hit. Recent findings have supported these predictions in considerable molecular detail.
Tumor in principal MEN1-related organ

Endocrine Tumors Expressed in 130 Patients with Tumor Patients, *

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Patients, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parathyroid tumor</td>
<td>129 (99)</td>
</tr>
<tr>
<td>Enteropancreatic neuroendocrine tumor</td>
<td>86 (66)</td>
</tr>
<tr>
<td>Gastrinoma</td>
<td>61 (47)</td>
</tr>
<tr>
<td>Insulinoma</td>
<td>15 (12)</td>
</tr>
<tr>
<td>Nonfunctioning tumor</td>
<td>5 (4)</td>
</tr>
<tr>
<td>Other</td>
<td>5 (4)</td>
</tr>
<tr>
<td>Pituitary tumor</td>
<td>61 (47)</td>
</tr>
<tr>
<td>Prolactinoma</td>
<td>34 (26)</td>
</tr>
<tr>
<td>Nonfunctioning tumor</td>
<td>14 (11)</td>
</tr>
<tr>
<td>Corticotropinoma</td>
<td>9 (7)</td>
</tr>
<tr>
<td>Somatotropinoma</td>
<td>4 (3)</td>
</tr>
<tr>
<td>Tumor in organ possibly related to MEN1</td>
<td></td>
</tr>
<tr>
<td>Carcinoid</td>
<td>21 (16)</td>
</tr>
<tr>
<td>Bronchial</td>
<td>11 (8)</td>
</tr>
<tr>
<td>Gastric</td>
<td>9 (7)</td>
</tr>
<tr>
<td>Thymic</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Adrenocortical tumor</td>
<td>21 (16)</td>
</tr>
<tr>
<td>Nonfunctioning</td>
<td>14 (11)</td>
</tr>
<tr>
<td>Functioning</td>
<td>7 (5)</td>
</tr>
<tr>
<td>Thyroid tumor</td>
<td>16 (12)</td>
</tr>
<tr>
<td>Follicular adenoma</td>
<td>10 (8)</td>
</tr>
<tr>
<td>Papillary carcinoma</td>
<td>6 (5)</td>
</tr>
</tbody>
</table>

* Each tumor type was scored only once per patient. MEN1 = multiple endocrine neoplasia type 1.
* Excludes multiple “nonfunctioning” islet tumors that were encountered incidental to each pancreatic operation for MEN1.
* Other = glucagonoma, somatostatinoma, and VIP (vasoactive intestinal peptide)oma.

Clinical Expressions of Multiple Endocrine Neoplasia Type 1 at the National Institutes of Health

Dr. Monica C. Skarulis (Division of Intramural Research, NIDDK, NIH): We reviewed patients with MEN1 who had been admitted to the NIH at least once in the past 15 years and had been evaluated by an NIH endocrine service. Clinical diagnosis of MEN1 was based on the finding of tumors in two or more of the three principal organs typically affected in the syndrome. A family history of MEN1-related tumor strengthened the diagnosis. A total of 107 patients (representing 58 families) had familial MEN1, and 23 other patients were designated as having sporadic MEN1 on the basis of a negative family history and negative biochemical family screening (where available).

Overall, the endocrine expressions in the familial and sporadic cases were indistinguishable from each other. Furthermore, affected family members varied widely in endocrine complications from MEN1. Only one family differed recognizably from the others in that its members had the pro lactinoma variant of MEN1.

All but one patient had parathyroid tumors (Table), which were multiple and often asymmetric. Because of the endocrinology patient referral biases of the NIH, this population is further enriched for hyperparathyroidism, the most highly penetrant and usually the first expression of MEN1 (1).

Enteropancreatic neuroendocrine tumors were identified in 66% of the patients. Gastrinomas of the duodenum and pancreas (with the Zollinger-Ellison syndrome) occurred in 47%. Insulinoma, occurring in 12% of patients, was generally a solitary, benign lesion. Rarely, a clinical syndrome associated with glucagonoma, VIP (vasoactive intestinal peptide)oma, or somatostatinoma was seen (Table). Nonfunctioning islet tumors detected by routine radiographic screening (computed tomography and magnetic resonance imaging [MRI]) are probably underreported in MEN1 because of the variable use and limited sensitivity of these techniques.

Pituitary tumors were seen in 47% of patients, and prolactinomas accounted for more than half of these tumors. The reported prevalence of pituitary tumors in MEN1 is 16% to 65% (11). Six members from one large family with the prolactinoma variant of MEN1, which is characterized by increased expression of prolactinoma and infrequent expression of gastrinoma (12, 13), were included in this series. Nonfunctioning pituitary adenomas, adrenocorticotrophic hormone (ACTH)–secreting adenomas, and acromegaly were more rare (Table).

Features with Weaker or Newly Recognized Association with Multiple Endocrine Neoplasia Type 1

Bronchial carcinoid was diagnosed in 11 patients (8%) by the presence of an enlarging chest lesion on radiography with or without pulmonary symptoms resulting from partial obstruction of a bronchus. Gastric carcinoid was detected incidentally on endoscopy in 9 patients with the Zollinger-Ellison syndrome (14). Bronchial or gastric carcinoids were often multifocal but were not clearly malignant, and one case of malignant thymic carcinoid was seen. Neither the carcinoid syndrome nor ectopic ACTH secretion (15) was seen in any of these NIH cases with foregut carcinoid tumor.

Tumors that have not been clearly proven to be a direct result of inactivation of the MEN1 gene and that have been only weakly associated with MEN1 include thyroid and adrenocortical tumors (16). Some two thirds of the adrenocortical tumors seen at the NIH were not associated with cortisol excess. In addition, three cases of renal angiomyolipoma and three cases of leiomyoma of the esophagus were found (17). One patient had a unilateral pheochromocytoma.

A recent analysis of patients with MEN1 seen at the NIH revealed frequent, subtle skin expressions. Multiple lipomas were found in 34% of patients with MEN1; multiple facial angiofibromas, which were previously considered pathognomonic for tuberous sclerosis, were found in 88%; and skin collagenomas were seen in 72% (18). The lipomas,
angiofibromas, and collagenomas showed 11q13 allelic loss (similar to loss of heterozygosity), which suggests that in MEN1, these are clonal neoplasms caused by inactivation of both MEN1 genes (19).

In addition, clinically apparent malignant transformation of enteropancreatic neuroendocrine tumors occurred, mostly in gastrinomas (nine cases) or nonfunctional islet-cell tumors (three cases), as well as in the single case of malignant thymic carcinoid mentioned earlier. Other cancers, probably unrelated to MEN1, included breast cancer (two cases), colon cancer (two cases), renal papillary cancer (one case), and uterine leiomyosarcoma (one case). Six cases of papillary thyroid cancer, all found incidentally on parathyroid exploration, were also noted.

**Age at Onset of Tumor Expression**

Although we did not prospectively screen for the development of new endocrinopathy, the peak age at onset of expression of endocrine tumors in patients with MEN1 (Figure 2) was about 20 years earlier than the age at onset of sporadic tumors in patients with hyperparathyroidism (20), gastrinoma (21), or insulinoma (22). By contrast, the age at onset of prolactinoma was indistinguishable between MEN1-associated and sporadic tumors (23).

**Tumor Localization in Multiple Endocrine Neoplasia Type 1**

Dr. John L. Doppman (Diagnostic Radiology Department, Clinical Center, NIH): The multiplicity of endocrine tumors in MEN1 can make surgical treatment difficult. At the NIH, generally available noninvasive methods, such as ultrasonography, computed tomography, MRI, and isotope scanning, have helped localize tumors before surgery in patients with MEN1. In addition, invasive methods, such as arteriography and venous sampling, that are specific to tumor type have been developed. Immunoassays have been applied to MEN1 tumors at the NIH; these assays test blood from selectively catheterized veins for parathyroid hormone or ACTH (the latter after systemic infusion of corticotropin-releasing hormone) or from a vein after infusion of a hormone secretagogue (calcium infusion for insulin release or secretin infusion for gastrin release). The following tumor localization guidelines derive from many institutions but mainly from the NIH.

**Parathyroid Tumor**

Patients with MEN1 and primary hyperparathyroidism almost always have parathyroid tumors in four glands. The appropriate first surgical procedure is a three-and-one-half-gland parathyroidectomy or a four-gland parathyroidectomy with immediate autotransplantation of parathyroid tissue to an accessible site (11). Because noninvasive studies do not effectively image parathyroid tumors in all four glands (24), tumor localizing studies are not indicated before initial parathyroid exploration. Sestamibi scanning detects more than 90% of parathyroid adenomas in patients who have not had surgery (25-27), but this technique images only about 60% of enlarged glands in multigland parathyroid disease.

Successful subtotal parathyroidectomy in MEN1 is often followed by recurrent hyperparathyroidism, characteristically within 15 years (28). Finding the recurrent tumor may require not only noninvasive localization studies (computed tomography, ultrasonography, MRI, and sestamibi scanning) but also parathyroid arteriography and selective parathyroid venous sampling for parathyroid hormone. The latter is particularly valuable in predicting multiple sites of residual parathyroid tissue, as well as the functional status of parathyroid autografts.

**Gastrinoma**

Gastrinomas associated with MEN1 are generally multiple and frequently extrapancreatic; the latter type occurs in the wall of the duodenum and in lymph nodes around the pancreatic head (1). Unlike sporadic gastrinoma, MEN1-associated gastrinoma is generally not cured by localized resection because of its multiplicity and frequent metastases (29). Because hyperacidity can be controlled medically with H2-receptor blockers or proton-pump inhibitors, the principal risk from MEN1-associated gastrinoma is liver metastases, which rarely occur until gastrinomas reach 3 cm (30). Therefore, our recommendation is to annually screen the abdomens of patients with gas-
crine neoplasia type 1. This patient had symptomatic hypergastrinemia. The adenoma, which was removed from the pancreatic head, stained for gastrin. Hyperinsulinemia remitted, and hypergastrinemia continued unchanged.

Insulinoma

In patients with MEN1, insulinomas are less common than gastrinomas (Table), but localization is a more frequent concern because medical control of endocrine effects is harder to achieve with insulinomas than with gastrinomas. Although all adults with MEN1 probably have multiple islet-cell tumors, MEN1-associated hyperinsulinemia, in our experience, is controlled by resecting a single adenoma (Doppman et al. Unpublished data). In contrast, hypergastrinemia in MEN1 is almost never controlled by resection of a single adenoma. The most successful test to localize tumors that oversecrete insulin is injection of calcium gluconate into selectively catheterized pancreatic arteries with sampling for insulin gradients in the right hepatic vein (32) (Figure 3).

Pituitary Tumor

Most pituitary tumors in MEN1 are prolactinomas (Table) that are successfully treated medically, although macroprolactinomas occasionally require transsphenoidal surgery because of their mass effect. The major pituitary tumor localization problem is ACTH-dependent hypercortisolism. Patients with MEN1 and the Cushing syndrome are rare, and although these patients with MEN1 may have non-pituitary tumors (pancreatic islet-cell tumors, bronchial carcinoid, or mediastinal carcinoid) that are theoretically capable of ectopic ACTH production, they almost always have a pituitary corticotropinoma (15). When gadolinium-enhanced MRI of the pituitary gland discloses a microadenoma, no further localization is required.

A patient with MEN1 and the Cushing syndrome whose MRI shows no pituitary tumor is treated like any patient with ACTH-dependent hypercortisolism and no demonstrable pituitary mass. Bilateral petrosal sinus sampling for ACTH, stimulated by ACTH-releasing hormone, is performed to unequivocally exclude ectopic ACTH production (33) and to provide lateralizing data that may be needed for transsphenoidal surgery. Such lateralization is correct in 70% of tumors and is the basis for performing hemihypophysectomy if no tumor is detected. Recently, intraoperative ultrasonography of the pituitary gland has further increased the accuracy of pituitary tumor imaging (34).

Several new endocrine tumor localization methods are helpful intraoperatively for MEN1 tumors of several organs. These include ultrasonography and rapid (on-line) hormone assay in blood or in tissue aspirates.

Role of Pedigree Linkage Analysis and DNA Maps in Localization of the MEN1 Gene

Dr. Francis S. Collins (Genetics and Molecular Biology Branch, National Human Genome Research Institute, NIH): In 1988, the MEN1 gene was mapped to the long arm of chromosome 11 (8) by means of the now-standard method of linkage analysis (35). For a highly penetrant Mendelian disorder such as MEN1, gene mapping is generally accomplished by collecting pedigrees of families with the disease and searching for associations with several heritable DNA sequences that serve as markers for nearby genes. If a marker is on a different chromosome than the disease gene, no allele of the marker will be associated with affected family members. With a large panel of markers (300 to 400), however, there is a good chance that one or more of them lie near the gene; coexpression of a marker and the disease is then to be expected. In that instance, the marker and the disease gene are on the same chromosome and are close enough that they are said to be linked. By observing exceptions (meiotic recombinations between the marker and the MEN1 trait) to that linkage in occasional members of pedigrees, it is possible to infer how close the marker lies to the disease gene. This process is generally iterated until one or more nearby markers with no recombination in all of the available families are recognized.

The original localization of MEN1 in 1988 was to
a very large region of chromosome 11 (8). Over the past decade, several groups refined the precision of this mapping by developing new markers and studying more families (13, 36, 37).

Physically Mapping the Candidate Interval of the MEN1 Gene

Once a candidate interval on chromosome 11 had been defined, it was desirable to obtain a subchromosomal road map (that is, an ordered set of cloned DNA markers that span this region) in order to look for candidate genes. Such a map of markers was made, along with a collection of many large human DNA fragments cloned into modified yeast or bacteria to span the entire MEN1 candidate region (38). This cloned human DNA also allowed the identification of additional polymorphic DNA markers (39), which could then be used with MEN1 pedigrees and endocrine tumors to further narrow the linkage-derived interval. By early 1997, through the work of several groups (40, 41), a genetic linkage-based MEN1 candidate interval of about 2 million base pairs had been defined (Figure 4).

Microdissection of Tumor Clones

Dr. Lance A. Liotta (Laboratory of Pathology, Division of Clinical Sciences, National Cancer Institute, NIH): To go beyond the narrowing that is obtainable through pedigree linkage analysis, the MEN1 candidate interval was narrowed further by analyzing DNA from endocrine tumors.

One possible expression of multiple neoplasia in MEN1 could be the development of hundreds of microscopic tumors in a patient with MEN1. Each of these could reflect an independent clonal second-hit event, bearing a different loss-of-heterozygosity zone on the normal copy of chromosome 11. If this happened, independent tumor clones could show unique loss-of-heterozygosity boundaries. All loss-of-heterozygosity intervals at chromosome 11q13 should still overlap the MEN1 gene, and a few loss-of-heterozygosity boundaries might establish a narrowed interval for MEN1 gene candidates (Figure 5).

The problem of tumor clone admixture with non-tumor cells is particularly notable in parathyroid tumors. Parathyroid glands in primary hyperparathyroidism show multiple areas of nodular and diffuse hypercellularity. Consequently, extracting total DNA from any macroscopic portion of the gland could encompass multiple neoplastic clones with a mixture of nonidentical second hits. Moreover, the tissues around and within the neoplasms can be highly vascular; thus, the neoplastic cells can be interlaced with stromal and other non-neoplastic elements, as shown by immunohistochemical staining for vessel markers. The heterozygous or unaltered allele from the contaminating normal cells (stromal or vascular) can thus obscure scoring of the lost allele from a tumor clone.

The use of extremely precise, controlled tissue microdissection can address the above three problems of adjoining tumor clones, admixture with surrounding normal cells, and admixture with interlaced normal cells. Microdissection was done with tapered pipettes and with laser-assisted cell capture; the latter was developed in the NIH Laboratory of Pathology (43–45).

Minimizing the MEN1 Candidate Interval from Data on Loss of Heterozygosity

In the late stages of the MEN1 gene discovery project, loss-of-heterozygosity zones on chromosome

---

Figure 4. Candidate interval for MEN1 gene. A. A map spanning approximately 2.8 million bases, part of the long arm of chromosome 11. DNA clones were assembled to span this entire interval. The minimal candidate intervals are shown for the location of the MEN1 gene, as determined separately from pedigree linkage or from loss of heterozygosity (LOH); the loss-of-heterozygosity–derived interval was far smaller. Genetic markers for chromosome 11, which by convention begin with D11, are shown above the line. Various expressed genes are shown by their names or abbreviated names below the line. Alpha, beta, and so forth are anonymous new genes assigned Greek letters as temporary names. B. A higher-resolution map featuring the area of maximum interest. A DNA sequence was available from three clones (C1, C2, C3 [C = cosmids]). Heavy horizontal bars below the map indicate the location of clones (b137C7, b79G17, and b18H3 [b = bacterial]), with inserts of human DNA, that were partially sequenced to search for candidate genes. The gene originally designated Mu turned out to be MEN1 (42). Cen = centromere; Tel = telomere.
Searching for Genes within a Candidate Interval

Dr. Francis S. Collins: Various strategies are used to find messenger RNA (representing expressed genes) in chromosomal regions of interest (46). These strategies include consulting already existing maps or using recombinant DNA methods that trap pieces of genes by their binding to cloned DNA taken from the candidate region. For regions consisting of fewer than 1 million base pairs, the method of choice has recently become direct sequencing of DNA across the entire interval. In some instances, the region is completely sequenced and assembled, leaving no gaps; in many others, however, a partial sequencing strategy is sufficient because it is likely to uncover sufficient evidence with which to identify most or all transcribed genes in the region. Thus, a major effort was undertaken to sequence three DNA clones that spanned the minimized MEN1 candidate interval (Figure 4B).

All of that sequence was assembled and then used to search the existing database of genomic sequences from all organisms, looking for any evidence of a match. Many candidate genes were uncovered (Figure 4) (42), some of them known genes that had not been previously mapped to a specific chromosomal location. Many, however, were identified only because they matched sequences in the human database of expressed sequence tags (short snippets of sequence from RNA). In most instances, a candidate gene sequence gave no clue about function; thus, the genes were assigned temporary names corresponding to Greek letters. Each of these genes then became a potential candidate for MEN1. Although some of the known genes seemed potentially more attractive because of hints of their biological function (such as a kinase or a zinc finger protein), none of them turned out to be the MEN1 gene, and it was necessary to carefully test most of the MEN1 candidate genes for mutations.

Germline and Somatic Mutations of the MEN1 Gene

Dr. Stephen J. Marx (Genetics and Endocrinology Section, NIDDK, NIH): A strong criterion for success in identifying a disease-causing gene is the demonstration of different germline mutations in different families with the disease. Germline DNA can be approximated by normal DNA, such as that from blood cells from lymphocytes, or from a buccal swab. This approach is particularly promising because most hereditary disorders, with some exceptions (such as MEN2 [2]), are caused by inactivating mutations that can occur at many different loci in the gene. Furthermore, most mutations are small

11q13 were determined in DNA from 188 microdissected tumors obtained from 81 patients with MEN1 tumors or sporadic endocrine tumors. Tumor types included parathyroid adenomas, pancreatic endocrine tumors, gastrinomas, and lung carcinoids. Six tumors (three MEN1-associated and three sporadic) provided key loss-of-heterozygosity boundaries (Figure 6). Four exhibited allelic loss only distal to marker PYGM. One showed loss that placed the MEN1 gene proximal (that is, away from the telomere) to marker D11S4936. This minimal overlap interval was encompassed by the loss-of-heterozygosity zone in 100% of the microdissected tumors that showed loss of heterozygosity at 11q13. The minimum overlapping region, bounded centromERICALLY (toward the centromere) by marker PYGM and telomERICALLY (toward the telomere) by marker D11S4936 (Figure 4 and Figure 6), became the new MEN1 candidate interval; this new interval was only 0.3 million bases long (45).
and are confined to the protein-encoding regions (the so-called open reading frame).

One MEN1 candidate gene that had not been previously cloned showed mutations in germline DNA from 14 of 15 MEN1 probands (47), conclusive proof of its identity as the MEN1 gene. This was subsequently confirmed in other families with MEN1 (48–51). Identifying this gene opened the possibility of testing for MEN1 mutations in related states and tumors as well.

**Germline MEN1 Mutation in Several States**

Among the 50 index cases from families with MEN1 evaluated at the NIH, 32 different MEN1 germline mutations were found in 47 cases (47, 48, 52) (Figure 7). No mutation was found in 3 families. Among the 50 kindreds, there was no correlation of mutation type with a disease phenotype, although the analysis included 3 large kindreds with a variant form of MEN1, termed MEN1Barlia or the prolactinoma variant of MEN1 (12, 13, 52).

In addition, the NIH analysis included eight cases of sporadic MEN1 (48) (defined as endocrine tumor in two of the three principal endocrine tissues affected by MEN1); 7 of 8 showed germline MEN1 mutation. This was subsequently supported by the finding among many familial and nonfamilial cases in the United Kingdom that 10% of MEN1 mutations arose de novo (58).

Several possible MEN1-like clinical disease states, or phenocopies, of a sporadic or hereditary nature might arise from the MEN1 mutation. Because primary hyperparathyroidism is the most frequent and usually the earliest endocrine expression of MEN1 (1), familial isolated hyperparathyroidism could be an incomplete expression of MEN1. Although five kindreds with familial isolated hyperparathyroidism were evaluated at the NIH, none showed germline MEN1 mutation; thus, it is more likely that familial isolated hyperparathyroidism is often caused by mutations in genes other than MEN1 (48). A comparable analysis in Japan of three kindreds with familial tumor of the pituitary also showed no MEN1 germline mutations (51).

### Somatic MEN1 Mutations in Endocrine Tumors

Several types of common endocrine tumors were tested for MEN1 mutations because of previous evidence from 11q13 loss-of-heterozygosity studies (1, 8–10). Studies from the NIH showed that MEN1 is the known gene most frequently mutated in many sporadic tumors, including parathyroid adenomas (21%) (53), gastrinomas (33%) (54), insulinomas (17%) (54), and bronchial carcinoid tumors (36%) (55). MEN1 is also mutated in a significant but small subset of sporadic pituitary tumors (5%) (56). For each sporadic tumor with MEN1 mutation, there was no corresponding mutation in paired germline DNA; the second copy of the MEN1 gene was also inactivated for each tumor, as evidenced by 11q13 loss of heterozygosity. These findings reaffirm the notion that the study of a gene contributing to rare hereditary tumors often uncovers important roles for the same gene in more common sporadic tumors.

### Deductions from MEN1 Mutation Patterns

The normal function of the MEN1 gene is unknown. In fact, its predicted protein ("menin") sequence shows no "signature" stretches that would predict known functions, such as membrane anchoring or DNA binding (48). Like the proteins encoded by about half of the known tumor suppressor genes, menin normally resides in the nucleus (59).

Most germline and somatic MEN1 mutations cause premature truncation of the predicted protein (57) (Figure 7), changes that would probably be catastrophic for menin bioactivity. The frequency of inactivating mutations found in germline or somatic DNA supports the theory of the first of two hits to a tumor suppressor gene (Figure 1).

**Figure 6.** Boundaries of loss of heterozygosity in endocrine tumors narrowed the chromosomal interval for the MEN1 gene hunt. Partial map of chromosome (Chr) 11q13 shows the relative locations of 16 polymorphic DNA probes. Data are diagrammed from 6 tumors (of 188) that provided informative markers. The region between D11S4936, and tumor 5 gave a narrower telomeric boundary at D11S4936. The region between PYGM and D11S4936, the minimal MEN1 candidate interval, is approximately 0.3 million bases (46). The same major landmarks are shown in Figure 4. Cen = centromere; Black squares = allelic retention; white squares = loss of heterozygosity at an informative marker.
Clinical Testing for Germline MEN1 Mutation

Although these discoveries at the gene level open the possibility of clinical genetic testing for MEN1 mutation, several issues will slow the development of MEN1 gene testing services in clinical laboratories.

1. Many different mutations. MEN1 mutations are spread across the nine exons of the predicted open reading frame (Figure 7), with novel mutations still being uncovered in most newly tested families with MEN1 (50, 51, 58).

2. No available testing shortcut. Because one third of mutations predict a change of one amino acid (missense) without truncation of menin protein, a so-called protein truncation shortcut assay would not detect any of the latter third.

3. Laboratory regulations. If their results will influence patient management, U.S. laboratories should comply with the regulations of the Clinical Laboratory Improvement Act (CLIA). Some laboratories do not yet have CLIA approval.

4. Limited demand. MEN1 is a rare trait, and testing for MEN1 germline mutation rarely leads to a major therapeutic intervention.

5. Counseling issues. A physician offering MEN1 gene testing must be prepared to provide or arrange pretest education, informed consent, and post-test counseling. A patient education booklet about MEN1 is available on the Internet at http://www.niddk.nih.gov/health/endo/pubs/fmen1/fmen1.htm.

Testing for MEN1 mutations differs in important ways from testing in patients with MEN2, for which RET gene testing can result in a major recommendation: thyroidectomy, often long before 18 years of age, to prevent or cure cancer (2). Cancer related to MEN1 has not been reported in patients younger than 18 years of age, and major morbidity from other expressions before this age is rare (1). MEN1 gene testing in persons younger than 18 years of age is rarely indicated because it will not lead to a major therapeutic intervention and because it would preclude an independent choice about testing when the child nears or reaches age 18, when appropriate understanding of the complex issues involved is more likely (60). More frequent morbidity before 18 years of age in patients with MEN1 could decrease the age at which testing is recommended.

Despite the above concerns, MEN1 mutation testing will probably soon be a clinical consideration. It is already possible, on a research basis, to identify the MEN1 germline mutation in most MEN1 kindreds. Clarification is also possible in sporadic (or even familial) disorders that incompletely resemble MEN1. Once the specific mutation is identified in an index case, it becomes technically far easier to test other relatives because the test can then be selectively directed at the known mutation. Those who test positive for the mutation can then be followed more closely for early signs of tumor expression. Those without the familial mutation can be spared from uncertainty and from potentially expensive prospective laboratory testing over many years.

The discovery of the MEN1 gene may promote future understanding of normal cell growth and of neoplasia, particularly in endocrine tissues. The gene will be subjected to intense study with powerful methods, and new therapies directed at certain sporadic or hereditary neoplasms could be developed from this knowledge.

Figure 7. Compilation of germline and somatic MEN1 mutations. Locations are shown on the horizontal bar containing MEN1 exons for germline MEN1 mutation in 56 kindreds (48-51) and for somatic MEN1 mutation in seven parathyroids, nine gastrinomas, two insulinomas, four bronchial carcinoids, and two pituitary tumors (53-56). Mutations shown above the exons cause protein truncation through stop codon or frameshift (small insertion or deletion), leading to a premature stop codon, two cause splice error. Those shown below the exons cause missense or one amino acid-codon change, by base substitution or by deletion of three bases without changing the triplet reading frame. Mutation descriptions follow standard nomenclature (57; see Glossary). bp = base pair; NIH = National Institutes of Health.
Glossary

Terms Related to Chromosomes

11q13: A subregion of chromosome 11 on the long arm (q arm) at cytologic band 1, subregion 3. The MEN1 gene is within this region.

Centromere: The one major constriction in a chromosome. The site of attachment to the mitotic spindle.

Distal and proximal: As applied to chromosomes, each chromosomal arm (short = p or long = q) has a distal portion (toward the telomere) and a proximal portion (toward the centromere).

MEN1: The MEN1 gene, whose mutation causes the MEN1 disease or trait. Genes and DNA loci (such as DUS4936) are italicized.

Telomere: Either end of any chromosome.

Terms Related to DNA Zones as They Change across Generations or through Tumorigenesis

Allelic loss: See loss of heterozygosity. Allelic loss has a broader definition because an allele can be recognized even if it does not have a polymorphic marker. Certain microscopic methods (for example, fluorescent in situ hybridization) can detect one compared with two or even more copies of a marker in a nucleus.

Germline DNA: DNA capable of passage to offspring. It can be present in the haploid state in a gamete or in the diploid state in gametogenic cells or in the totipotent cells of zygote. Blood DNA or normal-tissue DNA is often used to represent germline DNA of an individual person in studies of families or tumors.

Loss of heterozygosity: Marker loci with two different variants (alleles) in a germline are heterozygous. If there is allelic loss of one or more markers from a zone along one copy of the chromosome, all formerly heterozygous markers in that zone will have one remaining allele and thus will have lost the heterozygous state. Loss of heterozygosity is also called allelic loss. It implies loss of one copy of every gene fully contained in that zone and possible interruption of genes at the borders.

Polymorphic DNA marker: Segment of DNA from a mapped locus that shows variation from one individual person to the next and thus allows variants of the marker to be tracked in a family or during tumor progression. A synonym is DNA polymorphism. Most polymorphisms have no biological consequences.

Somatic DNA: DNA in postzygotic tissue, sometimes modified from the germline, that normally cannot be transmitted to the next generation.

Terms Related to Genes and Their Encoded Messenger RNA and Proteins

cDNA: Complementary DNA. DNA sequence copied from RNA or from DNA.

Exons and introns: DNA stretches in a portion of a gene that can be transcribed as premessenger RNA. The introns do not encode protein and are removed by splicing during maturation of messenger RNA. At the same time, the exons are spliced together as mature messenger RNA, which has a protein-encoding or open reading frame surrounded by regulatory elements at either end.

Mutation nomenclature: Amino acids are described by 1 of 20 single letters (not shown) with a number corresponding to their position in the amino acid sequence of a protein (Figure 7). The starting letter is the normal amino acid; the ending letter is the changed amino acid; thus, A309P changes amino acid 309 from alanine to proline. An amino acid change can be harmless (a normal variation or normal polymorphism) or harmful (a missense mutation). Mutations that create a stop codon are common and designated by X; thus, W183X changes amino acid 183 from tryptophan to a stop codon. Predicted stop codons are usually deleterious mutations. Some mutations change the triplet code reading frame (for example, base insertions or deletions that are not multiples of three) such that several inappropriate (nonsense) amino acids are read before a stop codon is encountered. These are designated by their nucleotide position in cDNA, followed by the change to the cDNA. For example, 1650insC is insertion of a cytosine nucleotide at cDNA position 1650. Most MEN1 mutations are small deletions, insertions, or substitutions, thereby affecting the open reading frame. Related conventions can be used to describe large deletions or changes within introns, particularly because the latter can change splicing sites.

Open reading frame: A portion of messenger RNA that encodes a protein sequence.

Acknowledgments: The authors thank major collaborators, including Sunita K. Agarwal, Mary Beth Kester, Christina Heppner, Young S. Kim, Paul K. Goldsmith, and A. Lee Burns (all from the National Institute of Diabetes and Digestive and Kidney Diseases [NIDDK]); Sirandashalli C. Gunn, Pachiappan Manickam, Shodimu-Emmanuel Olufemi, Judith Crabtree, and Settar A. Chandrasekharappa (all from the National Institute of Human Genome Research); Larissa V. Debelenko, Zhengping Zhuang, Irina A. Lubensky, and Michael E. Emmert-Buck (all from the National Cancer Institute); Mark Boguski and Jane Weisemann (both from the National Center for Biotechnology Information); Bruce Roe and Yingping Wang (both from the University of Oklahoma); and Jane S. Green (from Memorial University, Newfoundland, Canada). The authors also thank Lee S. Weinstein, William F. Simonds, and many current and former staff members and fellows in the NIDDK/National Institute of Child Health and Development Internist@Endocrine Fellowship Program and in the National Cancer Institute Surgery Branch.

Requests for Reprints: Stephen J. Marx, MD, Building 10, Room 9C-101, National Institutes of Health, Bethesda, MD 20892.

Current Author Addresses: Dr. Marx: National Institutes of Health, Building 10, Room 9C-101, Bethesda, MD 20892.
Dr. Spiegel: National Institutes of Health, Building 10, Room 9N-222, Bethesda, MD 20892.
Dr. Skarulis: National Institutes of Health, Building 10, Room 8S253, Bethesda, MD 20892.
Dr. Doppman: National Institutes of Health, Building 10, Room 10660, Bethesda, MD 20892.
Dr. Collins: National Institute of Human Genome Research, Building 49, Room 36-13, 49 Convent Drive, Bethesda, MD 20892.
Dr. Liotta: National Institutes of Health, Building 10, Room 2A33, Bethesda, MD 20892.

References


2. Farid NR, Buehler S, Russell NA, Maroun FB, Allerdice P.

3. Maton PN, Gardner JD, Jensen RT.

4. Fraker DL, Jensen RT.


6. 494 15 September 1998 •

7. Norton JA, Doppman JL, Jensen RT.

8. Rizzoli R, Green J 3d, Marx SJ.


