History and Epidemiology

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Medullary thyroid carcinoma (MTC) was not recognized as a distinct pathologic type of thyroid cancer until relatively recently. The correct classification of this thyroid neoplasm followed earlier histopathologic studies that identified a separate population of parafollicular or C-cells, the recognition that these cells had an embryologic origin from the neural crest, and the discovery that this cell population was responsible for the production of the peptide hormone calcitonin. Subsequent clinical studies established the strong familial patterns for the development of MTC and its association with specific hereditary cancer syndromes. The unique features of MTC include its intermediate level of biologic aggressiveness within the spectrum of thyroid cancer types, the production of a specific hormone product that can serve as a sensitive tumor marker for occult or recurrent disease (calcitonin), and its lack of susceptibility to radioiodine, making complete surgical removal of all cancer and lymphatic metastases the primary treatment goal. In addition, there have been great advances in the understanding of the molecular pathogenesis of MTC with the identification of germline-activating mutations in the *RET* proto-oncogene that are responsible for its development in association with the multiple endocrine neoplasia type 2 (MEN2) syndromes, as well as similar somatic mutations that underlie a significant proportion of sporadic tumors. Importantly, direct genetic testing allows for presymptomatic identification of patients who have inherited a disease-associated *RET* mutation and are at essentially 100 % risk of developing thyroid cancer during their lifetime. This ability to make a definitive genetic diagnosis prior to the detection of clinical, sonographic, or biochemical evidence of neoplasia allows the unique opportunity to perform an early thyroidectomy to remove the end organ at risk before invasive malignancy develops. Early prophylactic thyroidectomy for patients with MEN2 was one of the first and is perhaps still one of the best examples of a surgical intervention based on genetic testing that is intended to completely prevent subsequent cancer development in patients with an inherited cancer susceptibility.

History

Description of the Thyroid C-Cells

The story of MTC begins with the discovery of a separate population of cells dispersed in very small numbers within the thyroid parenchyma

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T.S. Wang and D.B. Evans (eds.), *Medullary Thyroid Cancer*,
DOI 10.1007/978-3-319-39412-1_1

that are embryologically, histologically, and functionally distinct from the thyroid follicular cell. In his communication to the Royal Society of London on January 27, 1876, entitled "Contributions to the Minute Anatomy of the Thyroid Gland of the Dog," E. Cresswell Baber first described this morphologically distinct population of cells [1]. Baber was studying the lymphatics of the gland and aptly declared during his preamble that the thyroid "is one of those organs, commonly known by the name of ductless or blood glands, about which our knowledge is still in a very unsatisfactory condition." Baber termed the separate population *parenchymatous cells*. These non-follicular cells were subsequently referred to descriptively as *parafollicular cells*, as proposed by Nonidez in 1932 [2]. This term is not entirely accurate, however, as the cells occur in both parafollicular and intrafollicular locations. The early studies of this cellular population were performed on animal thyroid glands, which have more prominent and larger numbers of "parafollicular" cells when compared to their human counterparts. José Fernandez Nonidez, an anatomist at Cornell Medical College in New York, performed histochemical studies as part of his work on the innervation of the thyroid gland of the dog. He utilized the reduced silver nitrate method of Cajal to characterize these cells based on their argyrophilic cytoplasmic granules. He recognized that these cells differed from the follicular cells in the thyroid glands of young puppies, in that they were larger and contained clear vesicular nuclei [3, 4]. Nonidez rediscovered Baber's "parenchymatous cells," and in discussing his elegant studies, he correctly proposed that the argyrophil granules represented "the antecedent of an endocrine secretion poured directly into the vessels." As reviewed by Roediger [5] and Hazard [6], these cells were recognized variously in subsequent studies of the mammalian thyroid as protoplasm-rich cells [7], ovoid cells [8], interfollicular cell [9, 10], neurohormonal cell [11], giant-light cell [12], mitochondrion-rich cell [13, 14], macrothyrocytes [15, 16], argyrophilic cell [17], gray cell [18], and stem cell [19]. The descriptive terms above were offered during precise early anatomic studies that focused on the morphology and histologic location of the cells within the thyroid gland. Pearse in 1966 [20] proposed the term C-cell, accurately indicating their function in the secretion of calcitonin.

The reasons for the late identification of this distinct cellular population in the human thyroid gland include their presence in very small numbers and non-specific appearance on routine histology. In the human thyroid, the C-cells exist singly or in small groups and are not easily discernible from the surrounding follicular cells unless special techniques are employed. On standard hematoxylin and eosin staining, the cells are slightly paler than the surrounding follicular cells but not sufficiently to provide positive identification. The most important historical staining method to identify the C-cells is the Grimelius silver nitrate argyrophil method [21], which results in characteristic, dark granular staining of the numerous secretory granules in the cytoplasm. Specific immunoperoxidase staining based on the presence of the hormone calcitonin was used in subsequent detailed studies of the distribution of C-cells in the human thyroid [22–24]. Ultrastructurally, C-cells are characterized by numerous round secretory granules that fill the cytoplasm, a well-developed Golgi complex, and moderate numbers of mitochondria. Electron microscopy studies have demonstrated that these cells are found in close proximity to follicular cells and within the follicular basement membrane. Thus, the "parafollicular cells" may occur in both a parafollicular and intrafollicular location.

The demonstration that these non-follicular cells synthesized, stored, and secreted calcitonin was the next major advance in the understanding of the thyroid gland's cellular structure and function. Copp and colleagues [25] in 1962 demonstrated the presence of calcitonin in canine thyroid–parathyroid preparations, but interpreted the source of the hormone to be the parathyroid glands. Foster [13] found that these "mitochondrion-rich" cells in the canine thyroid were responsive to hypercalcemia and proposed the name thyrocalcitonin to distinguish the "enzyme levels" from the hormone believed to be

produced by the parathyroid glands in the earlier studies by Copp. Bussolati and Pearse [26] subsequently demonstrated calcitonin in the C-cells of the porcine thyroid by direct immunofluorescent studies. Pearse [20] introduced the term "C-cells" to indicate the role of these cells in the production of calcitonin, which is most appropriate to describe their function. The C-cells belong to a dispersed family of peptide hormone-producing cells that Pearse [27] included under the descriptive term amine precursor uptake and decarboxylation (APUD) cells, based on common cytochemical and ultrastructural features, as well as his hypothesis that they shared a common embryological origin from the neural crest.

Dr. Anthony "Tony" G.E. Pearse was a pioneering pathologist and histochemist born in Kent, England, in 1916. He served as Surgeon Lieutenant during the Second World War and held an academic post in the Department of Morbid Anatomy (Histopathology) at the Hammersmith Hospital and the Royal Postgraduate Medical School of London, where he was appointed to Chair and first Professor of Histochemistry. Although subsequent studies demonstrated that some of the cells in the group did not derive from neural crest or even neuroectoderm, his pioneering work in expanding field of histochemistry, enzyme localization, and experimental embryology inspired a large number of scientists and contributed immeasurably to insight into the origins of the diffuse endocrine system and the biochemistry of peptide hormone-producing cells. He died in South Molton, Devon, on May 24, 2003.

The thyroid gland has a dual embryologic origin, originating from both the primitive pharynx and the neural crest. The main thyroid primordium develops as a midline thickening or proliferation of endodermal epithelial cells on the surface of the developing pharyngeal floor between the first and second pouches, in a location termed the foramen cecum. This proliferation begins as a hollow structure and later becomes solid and bilobed. The stem remains as the thyroglossal duct that does not descend into the lateral lobes. The rudimentary lateral thyroid anlage has origins from the pharyngeal endoderm and neural crest cells. The early ultimobranchial body arises from the pharyngeal endoderm and is subsequently populated by neural crest cells. The resulting ultimobranchial body gives rise to the C-cells. The derivation of parafollicular or C-cells from the neural crest was established by the experiments [28] with grafts of neural rhombencephalic primordium from 6 to 10 somite quail embryos implanted into the developing chick. The neural crest cells of quail have easily identifiable nuclear characteristics. Transplantation of these cells into non-quail neural crest was performed with the subsequent demonstration of cells with quail nuclei populating the ultimobranchial body in chick embryos, supporting the conclusion that C-cells arise from the neural crest and migrate during embryologic development. These cells associate embryologically with the ultimobranchial body, which is a ventral derivative of the fourth (or fifth) pharyngeal pouch. Some additional more recent studies, however, have suggested that revision of the concept of origin of the C-cells in mammals entirely from neuroectoderm may be necessary [29].

Anatomic studies have detailed the distribution of C-cells within the human thyroid gland. The C-cells have been shown to have an unequal distribution within different regions of the thyroid parenchyma. An early study of goitrous thyroid glands by Englund and coworkers [30] using argyrophil and fluorescent microscopy found that the extreme periphery of the lateral lobes just beneath the capsule and the isthmus was largely devoid of C-cells. In 1974, Wolfe et al. [24] and Tashjian et al. [22] mapped the distribution of C-cells in nonpathologic adult thyroid glands with detailed study of serial sections using immunoperoxidase localization and correlation of the histologic findings with radioimmunoassay for calcitonin in adjacent paired sections. These investigators concluded that the C-cells represent less than 0.1 % of the epithelial mass of the gland. The cells were shown to be primarily parafollicular, but also occurred in an intrafollicular location adjacent to the basement membrane and covered by a thin cytoplasmic

layer of the adjoining follicular cells which provided separation from the colloid within the follicle. These studies also demonstrated that C-cells are predominantly located within the deep portions of the middle and upper thirds of the thyroid lobe and are essentially absent in the isthmus. Wolfe et al. [23] performed a detailed study of six neonatal thyroid glands obtained immediately postmortem from normocalcemic subjects without associated endocrine disorders. They employed an immunoperoxidase localization technique with correlation by bioassay and immunoassay for calcitonin. In their study of neonatal thyroid glands, the C-cells were also distributed primarily in the middle to upper thirds of the lateral lobes. These cells were more numerous in the neonate than in adult (up to 75 C-cells per low power field) and more frequently intrafollicular in contrast to the findings from prior studies of the adult thyroid gland. The reason for the finding of increased numbers of C-cells in the neonatal thyroid was not clearly revealed in these studies, although it was speculated that the increased proportion might reflect the relative fraction in relation to the smaller gland volume, or alternatively that calcitonin might have an important role for the developing fetus. The anatomic distribution of C-cells within upper to middle thirds of the thyroid parenchyma corresponds to the most frequent location for the clinical development of medullary carcinoma within the gland.

Medullary Thyroid Carcinoma Arises from the Thyroid C-Cells

In his comprehensive contemporary 1977 review article "The C-Cells (Parafollicular Cells) of the Thyroid Gland and Medullary Thyroid Carcinoma" [6], Hazard attributes the first record of MTC as a specific pathologic diagnosis for a thyroid tumor to his unpublished observations in 1947. The solid cellular arrangement of the tumor type had previously resulted in its inclusion in the group of undifferentiated thyroid carcinomas, which were characterized by a highly aggressive malignant growth pattern. Dr. Hazard recognized a select subset of tumors with a characteristic "uniformity of cell type, a moderate number of mitoses as compared with the undifferentiated or anaplastic carcinoma, a uniform arrangement of cell sheets separated by stroma, and especially, the presence of amyloid in the tumor features that identified it as a distinctive histologic type."

Descriptions of malignant thyroid tumors containing amyloid have been found in the German literature from the early twentieth century, as cited by Ljungberg [31]. Horn in 1951 [32] published the findings from a series of 7 cases of a distinct variant of thyroid carcinoma with a moderate grade of malignancy and the gross and histologic features of medullary carcinoma, without description of the associated amyloid-containing stroma. A case report by Brandenberg in 1954 [33] characterized a thyroid tumor as "metastasizing amyloid struma," and Laskowski in 1957 [34] proposed the term "carcinoma thyroideum hyalinicum."

In 1959, a landmark paper by Hazard, Hawk, and Crile [35] from the Cleveland Clinic provided the first complete description of medullary (solid) carcinoma of the thyroid and identified this type of thyroid cancer as a distinct clinicopathologic entity. They reviewed the pathologic material from 600 carcinomas of the thyroid removed at the Cleveland Clinic Hospital between 1926 and 1957 and identified 21 cases with unique features warranting classification as a separate type of thyroid carcinoma. This report distinguished medullary carcinoma by its solid non-follicular histologic pattern, the presence of amyloid in the stroma, and a high incidence of lymph node metastasis. They noted that these tumors demonstrated an intermediate grade of malignancy, in distinction to the aggressive undifferentiated thyroid carcinomas with which they had been previously grouped.

John Beach Hazard was born on January 7, 1905, in White Horse, Pennsylvania. After completing his training at the Mallory Institute of Pathology, Boston City Hospital, he was a consultant in Pathology at the Robert Bent Brigham Hospital (1937–1946) and then ultimately chairman of the Division of Pathology at the

Cleveland Clinic Foundation (1958–1970). His many important contributions included the definitive first description of MTC and the original description of the presence of amyloid in the tumor. Known to his friends as "Beach," he had lifelong interest in horses and passion for horse racing and was regularly seen in attendance at the Kentucky Derby with his wife Mae. Dr. Hazard died in Key Biscayne, Florida, on September 13, 1994.

The origin of MTC from the C-cell was proposed by Williams [36] in 1966, and he suggested that the neoplasm was the source of a product with hypocalcemic activity. Meyer and coworkers provided support for this proposal through ultrastructural studies [37, 38] demonstrating the similarities of the cytoplasmic secretory granules of the tumor cells and C-cells, as well as the thyrocalcitonin-like activity exhibited by the tumor in tissue culture studies [38]. Subsequent additional studies [39, 40] confirmed the production of calcitonin by MTC tumor cells, and specific demonstration of calcitonin in the parafollicular C-cells was shown with the use of fluorescein-labeled antibody to calcitonin [41, 42]. The presence of an amorphous material in the stroma was a characteristic feature of the early descriptions of MTC, and this material was identified as amyloid. Albores-Saavedra et al. [43] identified amyloid fibrils by electron microscopy and confirmed that the amyloid material was a product of the MTC cells by tissue culture studies. Ultrastructural studies [37, 38] further characterized the amyloid fibrils and revealed that the formation of amyloid was predominately extracellular, but suggested that the secretory granules in MTC cells were involved in production of the material. Calcitonin was subsequently directly identified within the amyloid stroma by immunohistochemistry and immunofluorescence [44, 45]. Sequence analysis of the isolated amyloid medullary carcinoma of thyroid (AMCT) protein confirmed the presence of calcitonin, but with a higher molecular weight. This finding leads to the conclusion that an alternatively processed prohormone of calcitonin was a component of the amyloid material [45]. More recently, elegant mass spectrometry studies of the amyloid in MTC by Khurana et al. concluded that the full-length calcitonin was present [46], and mass spectrometry-based proteonomic analysis identified the peptides calcitonin and katacalcin [47]. Katacalcin is a member of the calcitonin gene family and flanks calcitonin in the human calcitonin precursor which is subsequently cleaved into calcitonin and katacalcin.

Peptide Hormones and Tumor Markers

Hirsch et al. [48] in 1964 purified a factor from extracts of porcine thyroid glands that was demonstrated to lower serum calcium and inorganic phosphate in rats. This agent, termed thyrocalcitonin, appeared to be a polypeptide that was distinct from thyroxine and triiodothyronine. The peptide hormone, later identified as calcitonin, assumed great importance as a tumor marker for MTC. Calcitonin plays a role in calcium homeostasis especially in lower animals, with a less prominent influence in humans. In common with other cells belonging to the dispersed neuroendocrine system derived from neural crest and the APUD cell concept of Pearse, the C-cells are capable of producing a variety of peptides, as well as biogenic amines, prostaglandins [49], and ectopic hormones such as ACTH [50], as reviewed in [51]. Baylin et al. [52, 53] demonstrated increased histaminase activity in the serum of patients with MTC and further reported that high levels of histaminase are present only in malignant C-cells suggesting that this activity might serve as a marker to distinguish microscopic carcinoma from C-cell hyperplasia. Elevated plasma levels of carcinoembryonic antigen (CEA) in patients with MTC were reported by Ishikawa and Hamada [54]. Wells et al. [55] studied 37 patients with MTC and demonstrated that serum CEA levels were elevated in 62 %, whereas serum calcitonin levels were elevated in 72 % of patients.

The finding that calcitonin is elaborated by normal C-cells and MTC tumor cells represented the next major advance in the diagnosis and clinical characterization of patients with this specific tumor type. The production of calcitonin

by C-cells and MTC tumor cells provides the ability to utilize circulating levels of this hormone as a sensitive tumor marker and proved to be a sensitive method to detect even small amounts of subclinical disease. Tashjian and Melvin [56] in 1968 reported their findings of a hypocalcemic agent, thyrocalcitonin, in studies of plasma and tumor extracts from two patients with medullary thyroid carcinoma. In the same year, they presented a case [40] of a thyrocalcitonin-secreting thyroid carcinoma with resultant hypocalcemia and "secondary hyperparathyroidism" from increased levels of circulating thyrocalcitonin. Further work described the clinical measurement of calcitonin and studies in patients, including the early diagnosis by means of the calcitonin immunoassay [57, 58].

The discovery that calcitonin secretion was stimulated by pentagastrin [59] and calcium [60] provided a sensitive clinical test to detect the presence of increased numbers of C-cells or occult carcinoma, even with very small numbers of neoplastic cells. Measurement of calcitonin response to secretagogues such as calcium and pentagastrin combined [61, 62] was employed clinically for the early diagnosis of MTC as well as to detect microscopic residual disease or follow patients for recurrence.

Familial Occurrence of Medullary Thyroid Carcinoma and Association with Specific Hereditary Endocrine Neoplasia Syndromes

The initial observation that eventually led to the recognition of the autosomal dominant inheritance pattern of hereditary cases of MTC, and its association with the specific MEN2 familial endocrine cancer syndromes, was the recognition that thyroid cancer occurred more frequently in patients with pheochromocytoma than would be expected by chance. Decourcy and Decourcy in 1952 [63] and Sipple in 1961 [64] noted an increased incidence of thyroid cancer in patients with pheochromocytoma. Sugg [65] recorded the interesting historical aspects Dr. Sipple's original observations based on direct personal interviews. Dr. John H. Sipple carefully documented the clinical findings in a case report of a 33-year-old index patient with both pheochromocytoma and carcinoma of the thyroid that he saw in a third-year medical student and later attended the patient's autopsy. Through an extensive literature review, he made the important observation that among 537 reported cases of pheochromocytoma, there were 5 additional cases also associated with thyroid cancer. Dr. Sipple was not an endocrinologist or a surgeon, but rather a pulmonologist. He graduated from Cornell University Medical College in 1955 and was a resident in internal medicine at State University of New York (SUNY) in Syracuse. After completing a fellowship in Pulmonary Disease at Johns Hopkins Hospital in Baltimore, he returned to SUNY where he was appointed Clinical Professor of Medicine in 1977. Sipple's observation of the association of pheochromocytoma with carcinoma of the thyroid gland is an early recognition of the dominant features of a specific genetic syndrome which eventually became Sipple's syndrome, or MEN2A.

Cushman subsequently recognized the association of pheochromocytoma, MTC, and parathyroid adenoma as a distinct clinical entity, in his 1962 report of a three-generation family with these tumors [66]. He noted that the specific type of thyroid carcinoma was medullary and correctly concluded that the syndrome was inherited in an autosomal dominant fashion. In 1965, Schimke and Hartmann described two families with inherited MTC, one with MTC only and one with MTC and pheochromocytoma, and identified amyloid-producing medullary carcinoma as the specific pathologic type of thyroid cancer with a strong familial association [67]. In that same year, Williams [68] reported the occurrence of thyroid carcinoma with pheochromocytoma in 17 patients, including 2 personal patients and 15 from the literature. In his report, Williams observed that MTC is the specific type of thyroid cancer associated with pheochromocytoma, the adrenal tumors may be bilateral, and that there is a frequent familial pattern of inheritance of these tumors. His report also recognized a few cases that involved

parathyroid tumors, raising the possibility of this pattern was related to a "multiple endocrine adenoma" syndrome. He emphasized, however, that this combination of endocrine tumors was distinct from the previously described syndrome of MEN1. A subset of Williams' cases also included multiple neuromas, and based on the neural crest origin of pheochromocytomas, he correctly surmised the possible neural origin of MTC. The complete description of the association and familial occurrence of MTC, pheochromocytoma, and parathyroid tumors as a distinct clinical entity was provided by Steiner et al. [69] in 1968, designating this constellation of findings MEN2. In this same year, a related syndrome including MTC, bilateral pheochromocytomas, and mucosal neuromas was described [70, 71], which was later recognized as MEN2B [72].

Genetic Basis for the Multiple Endocrine Neoplasia Type 2 Syndromes

A familial cancer trait is characterized by the occurrence of an unusual combination of otherwise rare endocrine tumors in a single patient, which is inherited from generation to generation. This pattern of clinical findings is ultimately the result of a disease-associated mutation in a specific human gene. In the 1980s, almost all of the human genetic sequence was uncharted territory and only a handful of human disease gene mutations had been identified. Prior to the human genome project, the only specific gene mutations that had been associated with human diseases had been identified either by first isolating the abnormal protein responsible, or in unusual circumstances where an obvious chromosomal abnormality gave a clue to the gene location (e.g., Duchenne muscular dystrophy and retinoblastoma). For researchers at the time, it was a formidable problem to search for and identify a disease mutation within the vast genome sequence, when the protein or gene location was not known. Genetic linkage analysis provided a way to follow the inheritance of markers within families and "map" a disease trait within the genome and to a specific chromosome region. The earliest human disease genes discovered were successfully identified through a laborious process termed positional cloning or "reverse genetics" [73], which involved searching for markers that are consistently inherited with the disease trait and using these signposts as a starting point to create a physical map of the disease gene region. "Chromosome walking" and "chromosome jumping" were strategies to cover associated areas [74, 75], but ultimately an exhaustive search of the nearby genes to uncover base-pair changes that only occurred in patients with disease was required. By 1990, only a few disease gene mutations had been identified, including genes for chronic granulomatous disease [76], Duchenne muscular dystrophy [77], cystic fibrosis [78, 79], retinoblastoma [80], and neurofibromatosis type 1 [81]. Identification of the specific gene mutations associated with familial adenomatous polyposis (FAP)/inherited colon cancer [82, 83], and inherited breast and ovarian cancer [84, 85] followed in the early 1990s.

The MEN2A disease gene was mapped by linkage analysis to the pericentromeric region of human chromosome 10 in 1987 [86, 87]. The three clinically distinct syndromes of MEN2A, MEN2B, and familial medullary thyroid carcinoma (FMTC) were shown to map to the same locus [88, 89]. Subsequent physical and genetic mapping studies focused the candidate interval to an approximately 500-kb region of chromosome 10q11.2 [90–92]. The MEN2 syndromes were ultimately shown to be associated with germline mutations in the *RET* proto-oncogene by two groups in 1993 [93, 94], thereby successfully identifying the disease gene. The rearranged during transfection (*RET*) proto-oncogene encodes a transmembrane receptor tyrosine kinase. *RET* mapped to the interval of interest on chromosome 10 and was known to be expressed in diverse tissue types including endocrine tissues, but allelic deletions or large chromosome aberrations (translocations, deletions, rearrangements) were not detected in tumor DNA from patients with *MEN 2*. The missense germline mutations associated with MEN2A were ulti-

mately identified by PCR amplification from *RET* genomic or cDNA and detection of sequence variants by single-strand conformational analysis or chemical cleavage mismatch procedure. The majority of these missense mutations occurred within one of several conserved cysteine residues in the extracellular domain immediately adjacent to the transmembrane portion. A single missense mutation in codon 918 within the intracellular catalytic domain of *RET* was shown to be associated with MEN2B [95, 96]. The occurrence of *RET* proto-oncogene mutations associated with the MEN2 syndromes was therefore within the earliest group of human disease genes identified by positional cloning.

Prophylactic Thyroidectomy for MEN2 Based on Genetic Testing

The ability to determine whether a patient has inherited a disease-associated germline *RET* mutation by direct DNA mutational testing provides the opportunity to intervene to completely remove the organ at risk for cancer development prior to its development. Early, prophylactic thyroidectomy can be performed based on genetic testing alone, before the development of biochemical or clinical evidence of MTC with the intent of eliminating the risk of subsequent neoplastic transformation and metastasis. Considering the three principle features of the MEN2 syndromes (MTC, pheochromocytoma, parathyroid tumors), MTC is the dominant clinical feature that is expected to develop in essentially 100 % of patients who inherit the mutation if followed for sufficient time. MTC is also the only consistently malignant component that accounts for almost all of the disease-related mortality. Because the end organ at risk for malignant transformation in MEN2, namely the thyroid gland, can be essentially completely extirpated at an early age by a meticulous and safe surgical procedure, this disease represents one of the first and perhaps still one of the best examples of a preventative surgical procedure performed for an inherited cancer susceptibility on the basis of a genetic test. Other notable examples include total proctocolectomy for FAP and bilateral prophylactic mastectomy/oophorectomy for patients with a *BRCA* mutation.

Skinner et al. [97] reported the long-term results of early prophylactic thyroidectomy in 50 children with a *RET* mutation identified through prospective genetic screening, with 100 % follow-up. A standard operation including total thyroidectomy, central cervical lymphadenectomy, and heterotopic parathyroid autotransplantation was performed in all patients by experienced pediatric and endocrine surgeons. All patients were evaluated patients for persistent or recurrent disease with basal and stimulated serum calcitonin levels. At 5 or more years postoperatively, 44 (88 %) of 50 patients had no evidence of disease based on this rigorous criteria. The rate of postoperative hypoparathyroidism was 6 %. Current guidelines include the performance of prophylactic thyroidectomy at approximately age 5 for patients with MEN2A and within the first years of life for patients with the more aggressive MEN2B syndrome (reviewed in [98]). For patients with mutations in one of the more infrequent, lower risk *RET* codons, thyroidectomy may be recommended at a later age.

Epidemiology

Thyroid cancer is the most common endocrine malignancy, representing roughly 96 % of endocrine cancer diagnoses and 3.8 % of all malignancies. There were approximately 62,450 new thyroid cancer cases and 1,950 deaths in 2015 [99–102]. Data from the Surveillance, Epidemiology, and End Results (SEER) program demonstrate that the overall incidence of thyroid cancer has been increasing, with the detection of new cases nearly tripling between 1975 and 2009, from 4.9 to 14.3 per 100,000 individuals [103, 104]. Using data from the National Vital Statistics System of the Centers for Disease Control and Prevention (CDC), it has been argued [104] that during the past nearly 35 years, the mortality rate from thyroid cancer has remained relatively stable (0.5 deaths per

100,000), leading to the conclusion that most of the increases in diagnoses represent small, subclinical papillary thyroid carcinomas with little clinical significance or impact on survival. However, others have argued that based on mortality data available from the SEER program, mortality from thyroid cancer actually increased approximately 1.2 % per year from 2001 to 2010 [99]. Notwithstanding a recent increase in thyroid cancer deaths, the incidence of follicular, medullary, and anaplastic thyroid carcinomas has remained stable during this time period [104, 105]. These less common thyroid cancer types are very infrequently detected as microcarcinomas. A major contribution to the observed marked increase in new thyroid cancer diagnoses over more than three past decades appears to be routine medical surveillance and improved detection of more subclinical tumors through widespread use of ultrasonography and fine needle aspiration of even small thyroid nodules [102, 106, 107]. However, there has also been an increase in the detection of large thyroid cancers (>5 cm) based on the SEER data from 1980 to 2005 [105]. Increased detection of small, clinically insignificant papillary cancers therefore likely does not explain all of the observed increase in thyroid cancer incidence. Stratification of the data by race [108] reveals that whites are experiencing the largest increase in overall age-adjusted thyroid cancer incidence (5.6 % per year), followed by blacks (4.8 % per year), American Indian/Alaskan natives (3.2 % per year), and Asians/Pacific Islanders (2.3 % per year). Non-Hispanics experienced a significantly greater increased incidence (5.5 % per year), compared with Hispanics (3.3 % per year).

Medullary thyroid cancer (MTC) comprises a small percentage of all thyroid cancer cases; the frequency in published reports ranges from 1 to 9 % over the last few decades [102, 107]. Despite the overall increase in thyroid cancer, the absolute incidence of MTC is likely relatively stable [107, 109]. A stable incidence of MTC with increased total thyroid malignancy (i.e., small papillary thyroid carcinomas) incidence may explain a relative trend toward a decreasing percentage of medullary cancers [106, 107, 109, 110].

Age

There are numerous factors that may contribute to the risk of developing MTC. These include age, gender, race, and genetic predisposition. In addition, the age at presentation, associated clinical characteristics, and timing of diagnosis and intervention influence prognosis and outcome in patients with MTC. The overall incidence of thyroid cancer (principally represented by differentiated papillary and follicular carcinomas) is known to have a midlife peak. MTC incidence, however, generally increases steadily with age and then tapers off somewhat in the extreme elderly. This age-related incidence pattern for MTC, stratified by gender, is illustrated in Fig. 1.1 (reproduced from [111]). Patients with hereditary MTC may be diagnosed in the first or second decade of life based on a program of biochemical screening in individuals at known risk, with an earlier distribution than for sporadic tumors. In a study by Kebebew [112], patients identified through genetic testing and/or prospective biochemical screening had a lower incidence of cervical lymph node metastases ($P < 0.05$) and were more likely to be cured at last follow-up when compared to patients with sporadic MTC not detected by screening (94.7 % vs. 49.4 %, $P < 0.0001$). Patients that underwent a total thyroidectomy and cervical lymphadenectomy were also more likely to be cured. In univariant analysis, age, gender, clinical presentation, TNM stage, sporadic/hereditary MTC, distant metastases, and extent of thyroidectomy were significant prognostic factors in patients with MTC. However, only age and stage remained independent prognostic factors in multivariant analysis.

Gender and Race

In keeping with other pathologic types of thyroid cancer, there is a gender predominance of females affected with MTC. However, this gender disparity is smaller in magnitude compared with differentiated thyroid cancers and continues to decrease with age [111]. There are modest

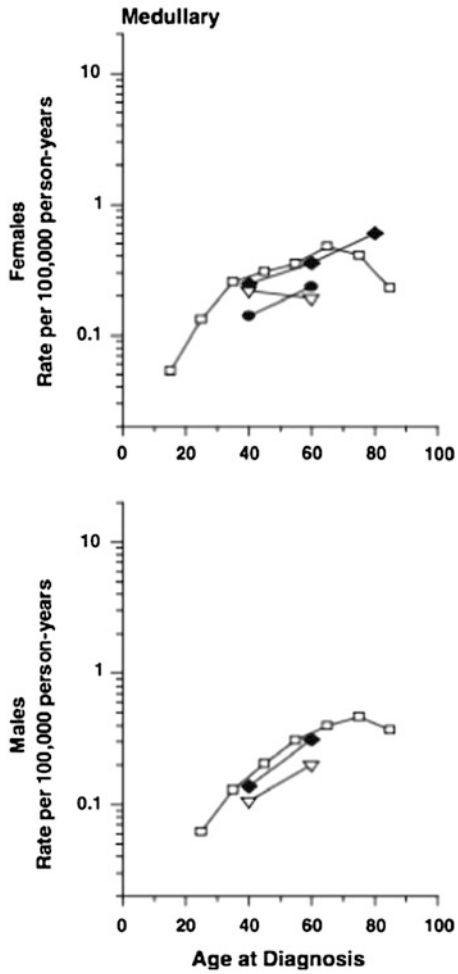

Fig. 1.1 Adapted and reproduced from Aschebrook-Kilfoy, 2011 [111]. Age-specific incidence of medullary thyroid cancer stratified by racial/ethnic group, SEER-13. Whites-*square* (□), Hispanics-*black diamond* (◆), Asians-*white down-pointing triangle* (▽), Blacks-*black circle* (●)

differences in the incidence of MTC according to race/ethnic background. In general, whites and Hispanics are more commonly affected than Asian/Pacific Islanders and blacks. Based on SEER data between 1992–2006, the overall incidence of thyroid cancer was 7.7 per 100,000 person-years. The highest incidence of MTC was in Hispanic and white women (0.21 and 0.22 per 100,000 woman-years). Among men, Hispanics had the highest rates (0.18 per 100,000 man-years) [99, 111] of MTC. Careful analysis of the SEER program data demonstrates uniformity of occurrence patterns between different geographic regions, arguing against socioeconomic disparities and access to health care as factors in the observed differences in incidence of MTC according to race and ethnicity [101, 111].

Sporadic Versus Hereditary MTC

MTC presents as both sporadic and hereditary disease. The majority (75 %) of cases are sporadic, while hereditary MTC occurs in approximately 25 % of diagnoses [111, 113]. Sporadic MTC is caused by somatic mutations in the *RET* proto-oncogene. Sporadic MTC commonly occurs in the fifth and sixth decades and demonstrates a slight gender preference toward female patients (1:1.13) [114–116].

Hereditary MTC is associated with the autosomal dominant inheritance of germline mutations in the *RET* proto-oncogene that are associated with *MEN 2* syndromes, with each child of an affected parent having 50 % risk of inheriting the gene mutation [109, 117–119]. Owing to the autosomal dominant inheritance pattern, males and females are affected equally with familial forms of MTC, in contrast to sporadic MTC. The inheritance of a disease-associated germline *RET* mutation confers essentially a 100 % risk of the subsequent development of thyroid C-cell malignancy if patients are followed long enough. Hereditary disease can be divided into clinically well-defined syndromes, reflecting the specific genotypic mutations responsible for the disorder. These clinical entities consist of *MEN 2A*, *MEN 2B*, and FMTC. The combined prevalence of the MEN2 syndromes is estimated to be between 1 in 30,000 and 35,000, with the majority of cases being represented by *MEN 2A*. In the USA, just under 500 cases of MEN2-related MTC are diagnosed annually [116].

MTC is the principle feature (greater than 95 % patients) of MEN2A and the consistently malignant component of the MEN2A syndrome. Hereditary MTC occurring in association with the MEN2A syndrome has an intermediate

Table 1.1 Classification and clinical features of medullary thyroid carcinoma

Variety of MTC and Incidence		Age at clinical diagnosis	Associated endocrinopathies
Sporadic—75 %		5th decade	None
Hereditary—25 %	MEN2A (~23 %)	3rd decade	Pheochromocytoma, parathyroid adenoma, cutaneous lichen amyloidosis
	MEN2B (~2 %)	1st decade	Pheochromocytoma, mucosal neuromas
	FMTC (rare)	Varied	None

Adapted from Friedhelm Raue and Karin Frank-Raue. Epidemiology and clinical presentation of medullary thyroid carcinoma [102]

aggressiveness, similar to that seen with sporadic MTC. The MTC that occurs in association with the rarer MEN2B syndrome (MTC, pheochromocytomas, marfanoid habitus, and multiple neuromas) is associated with a more aggressive biologic behavior conferred by the specific codon 918 *RET* mutation associated with this syndrome. Patients with MEN2B present with invasive MTC an early age, and late recognition of the syndrome and diagnosis of MTC results in a high frequency of metastatic disease in many patients.

FMTC is characterized by the autosomal dominant inheritance of MTC, without associated pheochromocytomas or parathyroid tumors [120]. The clinical characteristics of FMTC include the absence of pheochromocytoma or primary hyperparathyroidism and typically a later age of onset (after age 50 years) of MTC in multiple affected family members.

In summary, review of the available data on the epidemiology of MTC reveals that this distinct pathologic subtype represents a small percentage of thyroid cancer diagnoses. Overall, its incidence increases with age, and there is a slight female predominance. A summary of the clinical presentation of MTC is depicted in Table 1.1. The level of MTC cancer risk, age-of-onset, associated clinical manifestations, and recommendations for the extent of thyroid surgery and lymphadenectomy vary according to the specific *RET* mutation responsible for the MTC in a given patient [98]. The relationship of the underlying germline *RET* mutation to the aggressiveness of the MTC and the frequency of associated endocrinopathies is depicted in Table 1.2, as summarized in the revised American Thyroid Association Guidelines for the Management of Medullary Thyroid Carcinoma, 2015 [107]. It is estimated that up to 15 % of MTC is diagnosed post-resection on final surgical pathology, which may result in missed detection of pheochromocytoma and hyperparathyroidism and recognition of the familial inheritance risk, as well as an inadequate oncologic resection in some patients. These findings highlight the importance of heightened awareness for the early diagnosis and appropriate management of MTC [110].

Table 1.2 Relationship of common *RET* mutations to the risk of aggressive MTC in *MEN 2A* and *MEN 2B* and to the incidence of PHEO, HPTH, CLA, and HD in MEN2A (Reproduced from the Revised American Thyroid Associated Guidelines for the Management of Medullary Thyroid Carcinoma, 2015) [107]

RET mutation[a]	Exon	MTC risk levef[b]	Incidence of PHEO[c]	Incidence of HPTH[c]	CLA[d]	HD[d]
G533C	8	MOD	+	−	N	N
C609F/G/R/S/Y	10	MOD	+/++	+	N	Y
C611F/G/S/Y/W	10	MOD	+/++	+	N	Y
C618F/R/S	10	MOD	+/++	+	N	Y
C620F/R/S	10	MOD	+/++	+	N	Y
C630R/Y	11	MOD	+/++	+	N	N
D631Y	11	MOD	+++	−	N	N
C634F/G/R/S/W/Y	11	H	+++	++	Y	N
K666E	11	MOD	+	−	N	N
E768D	13	MOD	−	−	N	N
L790F	13	MOD	+	−	N	N
V804L	14	MOD	+	+	N	N
V804M	14	MOD	+	+	Y	N
A883F	15	H	+++	−	N	N
S891A	15	MOD	+	+	N	N
R912P	16	MOD	−	−	N	N
M918T	16	HST	+++	−	N	N

[a]The references for each of *RET* mutations can be found in the supplementary information, where all reported *RET* mutations in MTC are listed
[b]Risk of aggressive MTC: *MOD* moderate; *H* high; *HST* highest
[c]Incidence of PHEO and HPTH: + = ∼10 %; ++ = ∼20–30 %; +++ = ∼50 %
[d]*Y* positive occurrence; *N* negative occurrence

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