Multiple endocrine neoplasia type 2: recent progress in diagnosis and management

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Introduction

Multiple endocrine neoplasia type 2 (MEN-2) is an inherited endocrine disorder characterized by the occurrence of malignancies involving the C cells of the thyroid gland, the adrenal medulla, and the parathyroid glands (1). Medullary thyroid carcinoma (MTC) is the key component of MEN-2 and largely determines its mortality (2–6). MEN-2 is caused by germline mutations in the RET proto-oncogene and is transmitted to the offspring in an autosomal dominant fashion (7–17). Genetic identification of asymptomatic gene carriers susceptible to MEN-2 now allows the accurate diagnosis of MEN-2 in an early stage of the disease where prophylactic surgical treatment can be performed (9, 18, 19). Thus, MEN-2 has become the prototype of inherited endocrine disease in which genetic analysis leads to definite clinical decision making.

In this review, we summarize the classification, clinical features, diagnosis and treatment of this disorder. Furthermore, we discuss recent progress in the molecular basis underlying MEN-2, including genotype–phenotype relationships, and provide guidelines on how these genetic tests could be used for clinical management of affected patients or individuals at-risk from kindreds with MEN-2.

Clinical presentation

Classification

MEN-2 is subdivided into MEN-2A (the most common subtype), MEN-2B, and familial MTC (FMTC) (Table 1). MEN-2A is characterized by the triad of C-cell disease (C-cell hyperplasia or MTC), pheochromocytoma, and hyperparathyroidism (6). The International RET Mutation Consortium has proposed three phenotypic subtypes of MEN-2A (20). First, MEN-2A(1) in which all three components are present; secondly, MEN-2A(2) with C-cell disease and pheochromocytoma only, in the absence of hyperparathyroidism; and thirdly, MEN-2A(3) with C-cell disease and hyperparathyroidism only, in the absence of pheochromocytoma. In a rare variant form described in a few kindreds, disease components of MEN-2A were associated with the presence of cutaneous lichen amyloidosis (CLA), a circumscribed, highly pruritic skin disorder involving primarily skin between the scapulae and the extensors (21). CLA associated with MEN-2A has since been identified in 16 of 300 MEN-2A families studied and is most prevalent in patients of Oriental or South American heritage.

MEN-2B is characterized by C-cell disease, pheochromocytoma, ganglioneuromatosis, and a marfanoid body habitus (6). A variant of MEN-2B, with C-cell disease and prominent corneal nerves due to abnormal nerve myelination, but without typical ganglioneuromatosis, has also been described in a kindred (22).

FMTC is characterized by the presence of C-cell disease in at least four family members and the absence of parathyroid or adrenomedullary disease.

Disease components

The C-cell disease, present in almost 100% of patients with MEN-2, is usually detected before pheochromocytoma or hyperparathyroidism. Pheochromocytoma is present in 30–50% of patients with MEN-2A and MEN-2B. Hyperparathyroidism is present in 10–20% of MEN-2A patients.

The ganglioneuromatosis in patients with MEN-2B involves the lips, the anterolateral aspects of the tongue, the conjunctiva, and the gastrointestinal tract, the latter presenting with intermittent intestinal obstruction, gas formation, diarrhea, and failure to thrive. In contrast to Marfan’s syndrome, the marfanoid habitus associated with MEN-2B (tall stature, long limbs, pes cavum, pectus excavatum, and proximal muscle weakness) does not include any cardiac or ocular abnormalities such as mitral valve regurgitation, aortic dilatation, or dislocation of the lens.

Molecular basis

MEN-2A, MEN-2B, and FMTC represent incompletely penetrant inherited diseases that are transmitted in a dominant fashion with a 50% chance of inheritance while some generations may be spared. The susceptibility gene for these three disorders, and more recently
for Hirschsprung’s disease, has been located by linkage analysis to chromosome 10q11.2 where a candidate gene, the RET proto-oncogene, had been mapped (7). Subsequently, germline mutations in the RET proto-oncogene were detected in the majority of patients suffering from MEN-2A, MEN-2B, and FMTC (8–17), in a few patients with sporadic MTC (23, 24), and in some patients affected by Hirschsprung’s disease (25–27) (Table 2).

In MEN-2A, codon 634 is affected in the majority of cases (73–85% of kindreds), and the Cys → Arg mutation is the most common amino acid change in this codon (28–30). In MEN-2B, the amino acid change is exclusively Met → Thr in codon 918. In FMTC, codons 618 and 634 are the most affected codons.

The RET proto-oncogene contains 21 exons and encodes for a receptor tyrosine kinase which, similar to the receptors for insulin, insulin-like growth factor-I, epidermal growth factor, and platelet-derived growth factor, acts to autophosphorylate the cytoplasmic domain (31) (Fig. 1). Binding of the ligand causes dimerization and activation of the receptor. Unlike other susceptibility genes implicated in family cancer syndromes where both alleles need to be targeted, RET represents a proto-oncogene of which a single activating mutation of one allele is sufficient to cause neoplastic transformation (32). All mutations detected in patients with MEN-2 are activating and lead to gain of function (33). By contrast, RET proto-oncogene mutations associated with Hirschsprung’s disease are inactivating and lead to loss of function (27).

Expression of the RET proto-oncogene follows a distinct temporal and spatial pattern during embryogenesis with the enteric neuroblasts of the vagal crest and the nephric (Wolffian) duct being sites of consistent expression (34). The role of RET in embryogenesis has recently been highlighted by generating RET knockout mice which revealed absence of parasympathetic innervation of the gastrointestinal tract (35). In addition, RET-deficient mice displayed impaired nephrogenesis and urogenital abnormalities, but no abnormalities in endocrine organs affected in MEN-2 (35).

Recently, details on functional aspects of the RET proto-oncogene have been delineated. Mutations in codon 634, the most prevalent mutations in patients with MEN-2A syndromes, constitutively activate the receptor tyrosine kinase and subsequently lead to dimerization of receptor monomers, thus mimicking binding of the ligand to the receptor (33). Codon 918, located in a ‘pocket-shaped’ domain, is presumably implicated in substrate recognition of the receptor tyrosine kinase. Thus, mutations in codon 918 present in patients with MEN-2B act to constitutively activate the receptor tyrosine kinase by binding to and phosphorylating substrates other than receptor tyrosine kinases such as c-abl and c-src (36). Furthermore, mutations of codon 768, present in patients with FMTC, may alter kinase activity, possibly by changing substrate specificity or ATP binding capacity of the receptor (33). Finally, a distinct mutation in codon 804 (Val → Leu) alters binding specificity of the receptor which could activate the receptor by facilitating binding of unspecific ligands.

Studies on the structural–functional relationship of the RET proto-oncogene have been limited by the lack of a known RET receptor tyrosine kinase ligand. Recently, glial-derived neurotropic factor (GDNF), a member of the transforming growth factor-β gene superfamily, has been identified as a ligand for RET (37, 38). GDNF is a potent survival factor for central and peripheral neurons which is required during embryogenesis for the development of the kidneys and enteric neurons. GDNF-responsive cells express a glycosyl-phosphatidylinositol-linked protein (GDNF-α) which binds GDNF with a high affinity and mediates complex formation with the RET tyrosine kinase (39, 40). Intriguingly, GDNF-deficient mice display abnormalities similar to RET knockout mice, including abnormal kidney development and absence of enteric neurons, the latter

| Table 1 Classification of MEN-2. |
|-----------------|-----------------|
| Phenotype       | Disease component                              |
| MEN-2A          | MTC, pheochromocytoma, and hyperparathyroidism |
| MEN-2A(1)       | MTC and pheochromocytoma                        |
| MEN-2A(2)       | MTC and hyperparathyroidism                     |
| MEN-2A(3)       | MTC with or without pheochromocytoma, and ganglioneuromatosis and marfanoid habitus |
| MEN-2B          | MTC with or without pheochromocytoma, and ganglioneuromatosis and marfanoid habitus |
| FMTC            | MTC alone                                      |

| Table 2 Germline mutations in MEN-2. |
|-----------------|-----------------|-----------------|
| Phenotype       | Exon | Codon          |
| MEN-2A          | 10   | 609, 611, 618, 620 |
|                 | 11   | 634            |
| MEN-2B          | 16   | 918            |
| FMTC            | 10   | 609, 611, 618, 620 |
|                 | 11   | 634            |
|                 | 13   | 768            |
|                 | 14   | 804            |
resembling Hirschsprung’s disease (41–43). Future studies on the cross talks between GDNF, GDNF-α and RET and the generation of transgenic animals over-expressing GDNF, GDNF-α or RET will shed further light on the structure–function relationship of the RET proto-oncogene, and may reveal important novel insights into the molecular pathogenesis of MEN-2.

Pathology

MTC is grossly a solid, firm, whitish or yellowish tumor that usually lacks a circumscribed capsule. It is commonly located in the upper half of the thyroid lobe. The most common microscopical findings include trabecular, alveolar, and spindle cell patterns of tumor growth (44). Multinucleation, amyloid, and calcifications are also frequently present. The MTC is usually bilateral and multicentric, and may be in a background of C-cell hyperplasia, the precursor of MTC.

In contrast to sporadic pheochromocytoma, the adrenomedullary disease in patients with MEN-2 is commonly multicentric and bilateral. Similar to the incidence in non-MEN-2 patients, malignant pheochromocytoma is observed in less than 10% of cases (45, 46).

Hyperparathyroidism usually results from chief cell hyperplasia of multiple parathyroid glands, although a recent study reports single adenoma in the majority of cases (47).

Genotype–phenotype correlation

Specific mutations of the RET proto-oncogene considerably influence the phenotype. In MEN-2A, pheochromocytoma and hyperparathyroidism are more frequent in patients with codon 634 mutations as compared with those with other mutations (8, 12, 28–30). Of interest, according to Mulligan et al. (12), subjects with the subtype Cys → Arg mutation have an even higher frequency of hyperparathyroidism than those with other codon 634 subtype mutations. This finding, however, was not confirmed by others (8, 28, 30).

The presence of a distinct genotype can also influence the age at which disease components become clinically apparent. In MEN-2B (codon 918 mutation), MTC usually develops earlier than in MEN-2A and FMTC (other codon mutations). In MEN-2A, pheochromocytoma and hyperparathyroidism may develop earlier in patients with codon 634 mutations as compared with those with other mutations (30).

The basis of tissue specificity for RET mutations in tumorigenesis is currently unexplained. A tissue-specific threshold effect may exist whereby C cells and adrenal cells are most and parathyroid cells are least vulnerable to the growth-promoting effects conferred by the RET oncogene (12, 48, 49). The fact that phenotypic differences between MEN-2A and FMTC exist although individuals with these disorders may have identical RET mutations, suggests that other genetic and/or environmental factors may modulate the development of phenotypes in MEN-2.

Data on the relationship between RET mutations and the clinical outcome in MEN-2 are scarce. Codon 918 mutation is traditionally considered as an aggressive mutation with early development of metastases. For other mutations, in a preliminary study comparing the relationship of genotype to TNM stage in MEN-2A, no significant differences were observed between patients with codon 634 mutations and those with codon 618 mutations (50).

Screening

Genetic testing

Genetic screening is the most cost-effective and the least invasive method to detect asymptomatic gene carriers in at-risk family members of MEN-2 (18, 51–56).
Genetic testing should be performed very early in life, ideally shortly after birth. The test is performed using deoxyribonucleic acid isolated from peripheral blood leukocytes. RET proto-oncogene and/or linkage analysis are performed after polymerase chain reaction amplification (9).

In a kindred with a known mutation, members predicted not to be gene carriers do not need biochemical testing and no tests are required for their descendants. In contrast, gene carriers should be considered at high risk of developing C-cell disease (if not already developed).

In RET mutation-negative kindreds, linkage analysis may be helpful in identifying carriers. However, linkage analysis is not as reliable as RET mutation analysis because of errors due to recombinations (9). The possibility of sample mix-up or false paternity as well as de novo germline mutations should also be considered when the results of genetic testing are interpreted (24, 57, 58).

**Biochemical testing**

Primary relatives of MTC-affected patients with no identified RET mutation and with negative linkage analysis results require screening with biochemical tests.

Measurement of basal and stimulated plasma calcitonin (CT) levels is the method of choice for the diagnosis of MTC (59, 60). False-negative or false-positive pentagastrin stimulation tests may be observed in 5–18% of cases (9, 61).

Adrenomedullary disease is screened by annual measurement of urinary metanephrines and fractionated catecholamines (epinephrine, norepinephrine, dopamine), after the age of 6 years (45, 54, 60, 62). Patients with MEN-2 tend to have a higher epinephrine : norepinephrine ratio.

Primary hyperparathyroidism is screened by measurement of serum calcium levels every two years. Serum parathyroid hormone concentrations should be measured to confirm the diagnosis (54, 60).

The normal range for the above biochemical parameters varies between different laboratories. Table 3 summarizes current reference values at the Mayo Clinic Laboratories.

**Imaging techniques**

Other investigations including ultrasonography, chest X-ray, computerized tomography, magnetic resonance imaging, radionuclide scanning (201-thallium thallous chloride, 131-iodine meta-iodobenzylguanidine (MIBG), 99m-technetium hexakis 2-methoxyisobutylisonitrile (MIBI), and selective venous catheterization may be useful in the preoperative (as well as postoperative) evaluation of MEN-2. In particular, they may provide valuable topographic details in the assessment of the location and size of the primary tumor, particularly of ectopic parathyroid tumors and extra-adrenal pheochromocytomas, and of the presence and location of metastases (5, 63).

**Treatment**

Total thyroidectomy is recommended for all patients with C-cell disease or those at risk of developing it (6, 64, 65). Ideally, patients should be identified at the precancerous stage of C-cell hyperplasia. Subjects predicted to have inherited the RET mutation should have thyroidectomy at an early age (between the ages of 5 and 10 years for MEN-2A and FMTC, earlier for MEN-2B). Central compartment node dissection should systematically accompany the thyroidectomy (64–66).

Thyroid surgery should be undertaken after the screening for adrenomedullary disease in an experienced center by an experienced surgeon. If pheochromocytoma is discovered, adrenal surgery should precede thyroidectomy. Patients should undergo initial bilateral total adrenalectomy. Adrenal surgery must be preceded by the administration of appropriate alpha- and beta-adrenoceptor blockers (phenoxybenzamine hydrochloride and propranolol hydrochloride) as well as generous fluid and electrolyte repletion.

Treatment of hyperparathyroidism consists of subtotal parathyroidectomy with preservation of a well vascularized portion of a single gland in situ or its transplantation to the non-dominant forearm.

**Follow-up and prognosis**

All patients should receive lifelong replacement therapy with thyroid hormone (levothyroxine sodium, orally, 100–150 μg/day in adults) after thyroid surgery. Serum thyrotropin concentration should be maintained within the normal range and, in contrast to non-medullary thyroid carcinoma, does not need to be suppressed. Follow-up of these patients is mainly based on basal (only if high) and stimulated plasma CT determinations. Therapy of patients with metastatic MTC is primarily surgical (67). Radiotherapy is indicated for skeletal metastasis or non-resectable metastatic MTC. The
clinical course of patients with MTC varies with tumor characteristics. Overall, 10-year survival rates are approximately 65%. Age at onset, stage and completeness of initial surgical resection are among the most significant prognostic factors in the outcome of patients with MTC (68–70).

Patients with bilateral adrenalectomy should receive lifelong replacement therapy with appropriate glucocorticoid (hydrocortisone, orally, 20–30 mg/day in adults) and mineralocorticoid (fludrocortisone acetate, orally, 100 μg/day in adults) therapy. Repeat measurements of catecholamines will detect recurrent or metastatic pheochromocytoma, especially if the patient has had unilateral adrenalectomy.

Postoperative hypoparathyroidism is treated with calcium (calcium citrate, orally, 1 g/day elemental calcium in adults) and vitamin D (calcitriol, orally, 0.5–2 μg/day in adults). Patients who have not yet developed signs of pheochromocytoma or hyperparathyroidism should be screened at regular intervals. This is particularly important in patients with codon 634 mutations who are more prone to develop these components (29, 30, 71).

Conclusion

The discovery of the molecular basis of MEN-2 and recent findings derived from animal models deficient in RET and GDNF have greatly enhanced our knowledge of the functional aspects of the RET proto-oncogene. MEN-2 has since become an inherited endocrine disease in which genetic testing can identify almost all affected individuals at an early presymptomatic stage. Asymptomatic gene carriers of hereditary MTC should have prophylactic total thyroidectomy regardless of the plasma CT levels. Thus, early diagnosis based on genetic testing and subsequent treatment may considerably improve the prognosis of this disorder. The knowledge of the particular mutations in MEN-2A kindreds may also be helpful in the follow-up with biochemical testing for the early detection of other components of the disease.

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