

Review**Genotype-phenotype relationship in multiple endocrine neoplasia type 2. Implications for clinical management**

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Multiple endocrine neoplasia type 2 (MEN2) is an autosomal dominant tumour syndrome caused by germline-activating mutations of the *RET* proto-oncogene. It has a strong penetrance of medullary thyroid carcinoma (MTC) and can be associated with bilateral pheochromocytoma and primary hyperparathyroidism (MEN2A) within a single patient or family. Based on the phenotype three distinct clinical forms have been described: (1) classical MEN2A, (2) MEN2B, an association of MTC, pheochromocytoma and mucosal neuroma and (3) familial MTC (FMTC), which is associated with a very low incidence of other endocrinopathies. Each variant of MEN2 results from a different *RET* gene mutation, with a good genotype-phenotype correlation with regard to aggressiveness of MTC, time of onset of MTC and the presence or absence of other endocrine tumours. Recommendations on the timing of prophylactic thyroidectomy and extent of surgery are based on a classification of *RET* mutations into three risk levels using the genotype-phenotype correlations. MEN2 provides a unique model for early prevention and cure of cancer and for stratified roles of mutation-based diagnosis of carriers.

Keywords: Medullary thyroid carcinoma, Multiple endocrine neoplasia type 2, Pheochromocytoma, Primary hyperparathyroidism, *RET* gene mutation

A. INTRODUCTION

Multiple endocrine neoplasia type 2 (MEN2) (OMIM 171400) is an autosomal dominant syndrome with an estimated prevalence of 2.5 per 100,000 in the general population. Predisposition to MEN2 is caused by germline activating mutations of the *RET* proto-oncogene.^{1,2} MEN2 shows a high penetrance for medullary thyroid carcinoma (MTC), a rare calci-

tonin (CT)-secreting tumour of the parafollicular or C-cells of the thyroid. Most patients suffer from the sporadic (non-familial) form, while 25-30% account for hereditary MTC. Hereditary MTC is usually bilateral and multicentric; multifocal C-cell hyperplasia is considered to be a precursor of MTC in patients with hereditary disease. Although MEN2 syndromes are rare, recognition is important both for treatment and for genetic counselling.

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CLINICAL SYNDROMES OF MEN2 (Table 1)

MEN2 syndrome occurs in three clinically distinct varieties with MTC as the most common manifesta-

Table 1. Clinical Classification of MEN2 and occurrence of Medullary Thyroid Carcinoma (MTC), associated tumours and other diseases

Subtype	Percent(%) of total cases	MTC %	PHEO %	HPT %	Associated diseases
MEN2A	56	100	50	25	Cutaneous lichen amyloidosis, Hirschsprung's disease
MEN2B	9	100	50		Ganglioneuromatosis, marfanoid habitus
FMTC	35	95			Very rare

PHEO: pheochromocytoma, HPT: hyperparathyroidism,

tion. The three subtypes of MEN2 differ with respect to incidence, genetics, age of onset, association with other diseases, aggressiveness of MTC and prognosis.^{1,2}

MEN2A SYNDROME

MEN2A syndrome is the most common form and is characterized by MTC in combination with pheochromocytoma and/or multiple tumours of the parathyroid glands in a single patient, or the presence of two or more tumour types in multiple members of a single family.² Among patients with MEN2A, the frequency of MTC is over 90%, of pheochromocytoma 40-50%, and of multiple parathyroid gland hyperplasia or a single adenoma 10-20%. MTC is generally the first manifestation of MEN2A and develops between the ages of 5 to 25 years. Some rare variants of MEN2A exist, including MEN2A associated with cutaneous lichen amyloidosis, and FMTC (or MEN2A) with Hirschsprung's disease.

MEN2B Syndrome

MEN2B syndrome is the least common but the most aggressive form of MEN2 (5-10% of all cases). It consists of MTC, pheochromocytoma, without hyperparathyroidism, and visible physical stigmata like bumpy lips and tongue (neuromas) and a marfanoid habitus. Patients with MEN2B typically experience disease onset in the first year of life and have a more aggressive form of MTC with higher morbidity and mortality rates compared with patients with MEN2A.

Patients with MEN2B also have mucosal neuromas, intestinal ganglioneuromas and a marfanoid habitus with skeletal deformations and joint laxity but without the vascular and ophthalmologic abnormalities. They often do not have a family history of the disease and harbour a de novo mutation.

FMTC Syndrome

Familial MTC (FMTC) is the mildest variant of MEN2. It has been diagnosed more frequently in recent years (35-40% of all cases).³⁻⁵ In patients with FMTC, there is a strong predisposition to develop MTC with a very low incidence of the other clinical manifestations of MEN2A. The diagnosis of FMTC can only be considered when four or more family members across a wide range of ages have isolated MTC. The clinical course of MTC in FMTC in general is more benign than for individuals with MEN2A and MEN2B and typically has a late onset or no clinically manifest disease. The prognosis for FMTC is relatively good; however, aggressive MTC tumours and even death due to MTC have been reported in cases harbouring codon 804 mutations. A family history is often inadequate in establishing diagnosis of familial disease, a more thorough evaluation by genetic and biochemical screening often revealing a family history of MTC in patients originally thought to have the sporadic form of the disease.

RET PROTO-ONCOGENE: STRUCTURE, FUNCTION AND MUTATIONS

The *RET* gene has 21 exons and encodes a tyrosine kinase receptor that appears to transduce growth and differentiation signals in several developing tissues including those derived from the neural crest. The protein consists of an extracellular part with a ligand-binding domain, a cadherin (Ca²⁺-dependent cell adhesion)-like domain and a cysteine-rich domain close to the cell membrane. It has a single transmembrane domain and an intracellular part with two tyrosine kinase subdomains, TK1 and TK2. The RET protein is activated upon ligand-induced dimerization.⁶ *RET* is expressed in neuroendocrine cells such as C-cells, the precursors of MTC, and in pheochromocytomas. Hereditary MTC is caused by autosomal dominant gain-of-function mutations in the *RET* proto-oncogene.

The MEN2 gene was localized to centromeric chromosome 10 (10q11.2) by genetic linkage analysis in 1987. Subsequently, point mutations of the *RET* proto-oncogene were identified in MEN2A, MEN2B and FMTC in 7 exons located nearby (exons 8, 10, 11, 13-16).^{7,8} Analysis of *RET* in families with MEN2A and FMTC revealed that nearly 100% of these families have germline mutations and that only those family members with the germline missense mutation had the disease. This discovery prompted major advances in our understanding of the molecular genetic basis of MTC and has significantly changed the clinical management of families with hereditary tumours. At present, mutation analysis in MEN2 families has identified over 50 different missense mutations segregating with the disease.⁵

Mutation of the extracellular cysteine at exon 11 codon 634 causes ligand-independent dimerization of receptor molecules, enhanced phosphorylation of intracellular substrates and cell transformation. Mutation of the intracellular tyrosine kinase (codon 918) has no effect on receptor dimerization but causes constitutive activation of intracellular signalling pathways and also results in cellular transformation. There is a significant age-related progression from C-cell hyperplasia (CCH) to MTC, which correlates with the transforming capacity of the respective *RET* mutations.⁹ MTC is generally the first neoplastic manifestation because of its earlier and higher penetrance compared with pheochromocytoma or parathyroid hyperplasia. This indicates that C cells are more susceptible to oncogenic *RET* activation than adrenal medullary or parathyroid cells.

CHANGE IN THE SPECTRUM OF DETECTED *RET* MUTATIONS OVER THE LAST TWO DECADES

Our analysis of the *RET* proto-oncogene in patients with hereditary MTC, diagnosed between 1994 and 2006, provided evidence for a change in the spectrum of detected mutations: increase of diagnosed cases from 10% to 39% of the families with mutations in exon 13-15, so called "rare mutations" (Figure 1).⁵

This change in the frequency of diagnosed mutations in families with classical MEN2A from mutations mainly in exon 11 codon 634 (formerly 85% of

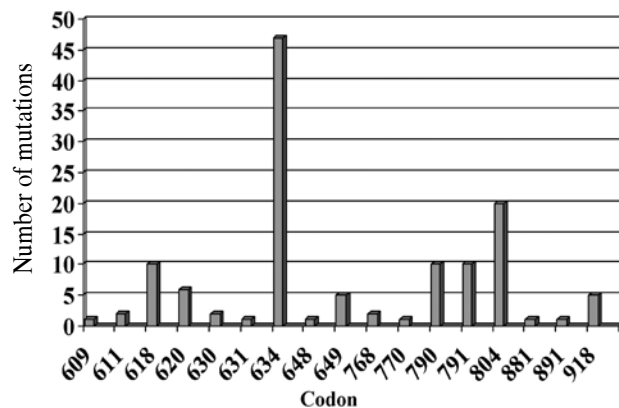


Figure 1. Distribution of different *RET* mutations (130 families) identified between 2000 and 2008 (Laboratory of Molecular Genetics, endocrine practice, Heidelberg).

all MEN2A mutations) to the so-called rare mutations localized at codons¹⁰ 13, 14 and 15 may also have impact on the overall prognosis of hereditary MTC. The reasons for this change in the spectrum may be attributed to the improvement in diagnostics, since *RET* mutations are routinely looked for in all patients diagnosed with MTC, not only familial but apparently sporadic cases as well (4-7% hereditary). Furthermore, analysis involves not only known hot spots but other known mutations.⁴ It must be stressed that there is a distinct distribution of *RET* mutations in different parts of the world depending on the genetic background.

GENOTYPE-PHENOTYPE CORRELATION IN MEN2

Clear associations between specific *RET* mutations (genotype) and the age at onset and aggressiveness of MTC and the presence or absence of other endocrine neoplasms (phenotype), such as pheochromocytoma or hyperparathyroidism, are well documented.^{10,11} Although some overlap exists between *RET* mutations and the resulting clinical subtype of MEN2, 85 % of patients with MEN2A have the codon 634 (exon 11, cystein-rich domain) mutation, mutations of codon 609, 611, 618 and 620 accountings for a further 10% to 15%. Pheochromocytomas are also detected in about 50% of patients with 634 and 918 codon mutations and are rarely seen in mutations of exon 10 (codon 609, 611, 618, 620) or exon 15 (codon 791, 804) (Figure 2).^{12,13} Hyperparathyroidism in

MEN2A is most commonly associated with codon 634 mutations, and in particular with the C634R mutation¹⁴ (Figure 3).

The association between disease phenotype and *RET* mutation genotype may have important implications for the clinical management of MEN2 patients and their families. If the genotype can be fully correlated with certain phenotypic features, then a clinician could use a patient's genotype to decide whether to intensify screening for pheochromocytoma or hyperparathyroidism for those patients with mutations associated with a higher risk.

All cases of MEN2A with Hirschspung's disease have mutations in exon 10 (codon 609, 611, 618, 620), while patients with MEN2A and cutaneous lichen amyloidosis are associated with mutations in codon 634.

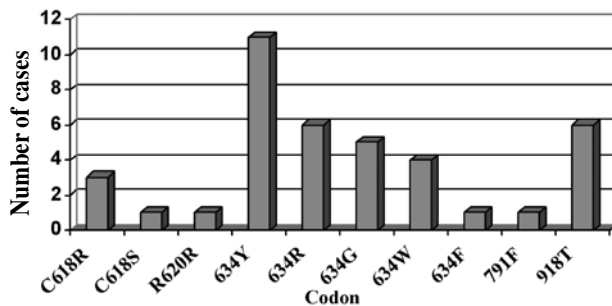


Figure 2. Pheochromocytoma in MEN2. The distribution of patients with pheochromocytoma in the different *RET* mutations (endocrine practice, Heidelberg) in a series of 130 families with *RET* mutations.

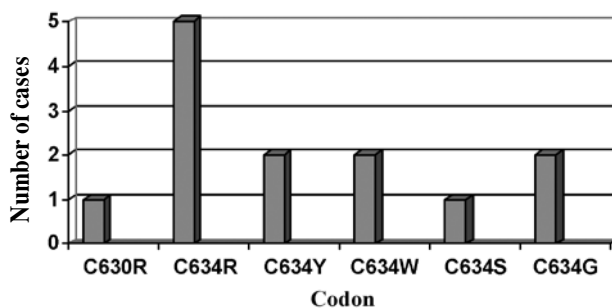


Figure 3. Primary Hyperparathyroidism (HPT) in MEN2. Distribution of patients with HPT in the different *RET* mutations (endocrine practice, Heidelberg) in a series of 130 families with *RET* mutations.

More than 95% of MEN2B patients have mutations in codon 918 (exon 16, tyrosine kinase domain) and, rarely in codon 883 of exon 15. The 918 mutation results in an ATG (methionine) to ACG (threonine) alteration, which is significant since Met 918 is a critical component of the substrate recognition pocket in the tyrosine kinase catalytic core of the RET protein.

In FMTC, germline mutations are distributed throughout the *RET* gene with an accumulation in exon 13 (codon 768, 790, 791) and exon 14 (codon 804,844). Some of these mutations have also been identified in families with MEN2A. Because FMTC shares a common genetic defect with MEN2A, it can be difficult to distinguish a family that initially appears to be FMTC from MEN2A, as the manifestation of pheochromocytoma and/or hyperparathyroidism occurs over time.

PATIENT RISK GROUPS

Extensive literature shows a correlation between the specific germline *RET* mutation and the age of onset and aggressiveness of MTC development and nodal metastases.⁹ Patients with codon 918 mutation and MEN2B have a high risk for aggressive MTC at young age. In contrast, patients with codon 791 mutations have a relatively low risk of a late manifestation and slowly growing tumour.¹⁵ This genotype-phenotype correlation of age at onset and aggressiveness of MTC is the basis for decision making in clinical management, especially in pre-symptomatic *RET* mutation carriers, as prophylactic thyroidectomy has to be performed prior to the development of cancer. This strategy for prevention of familial MTC should be tailored to the specific mutation carried by the individual patient.

Recommendations for the timing of prophylactic thyroidectomy and the extent of surgery are based upon a model that utilizes these genotype-phenotype correlations to stratify mutations into three risk levels.^{16,17} Patients with level 1 mutations (codons 609, 768, 790, 791, 804 and 891) have a high risk for MTC development and growth, patients with level 2 mutations (codons 611, 618, 620 and 634) are higher risk patients, and patients with level 3 mutations (codons 883 and 918) are at the highest risk for early development and growth of MTC (Table 2).

Codon-specific prognosis would facilitate individual risk stratification for each patient. Furthermore, good evidence exists of a significant age-related progression from C-cell hyperplasia (CCH) to MTC that correlates with the transforming potential of level 2 (codon 634) and level 3 mutations (codon 918).⁹ In patients with the higher risk mutations (level 2), a thyroidectomy is recommended at the age of five years, and as early as possible (preferably in the first year after birth) for patients with level 3 mutations.¹⁶ For patients with level 1 mutations there are three alternatives concerning recommended age at prophylactic surgery: some authors suggest 5, others 10 years of age, others, as the authors do,¹⁵ suggest that surgery may be postponed until after an abnormal C cell stimulation test result is observed (i.e. an abnormal CT response to pentagastrin or calcium stimulation). Further studies, especially in the rare mutations,¹⁸ are necessary before more specific recommendations can

be made (Table 3).

In recent years a certain variability in clinical presentation among genetically related individuals has been observed. This intrafamilial phenotypic variability regarding the age at onset and disease extent at diagnosis (tumour size, presence of lymph nodes or distant metastases) is a matter of further investigation in this field. It has been speculated that interacting genetic alterations may be responsible for this phenotypic heterogeneity. Single Nucleotide Polymorphisms (SNPs) within the *RET* oncogene have been reported (G691S, L769L, S836S, and S904S) and the modifying role of the clinical phenotype of MEN2 have been discussed.^{19,20} Only the *RET* G691S variant could modulate the age of onset of familial and sporadic MTC with higher basal calcitonin levels.²¹ We have confirmed the data of Cardot-Bauters²¹ in our series of 66 patients with hereditary MTC with regard to the aggressiveness of the MTC; we found that tumour stage increase was related to the frequency of G691S variants in patients with MEN2 (Figure 4).

Table 2. Codon-based genotype-phenotype correlation.

Risk of MTC development	Mutated codon	MEN2 subtype
High (level 1)	609,649,768,791,790,804,891	FMTC, MEN2A
Higher (level 2)	634, 611,618,620,630,631	MEN2A
Highest (level 3)	918, 883	MEN2B

PERSPECTIVES

The biological behaviour of MTC can now be stratified by mutations of the *RET* proto-oncogene, which guides the timing of prophylactic thyroidectomy and the extent of surgery. This has to be established for

Table 3. Management of patients with different *RET* Mutations

Codon	609,791,790,804, 649,891,768	634 611,618,620 630,631	918 883
Risk level	1	2	3
MEN2 subtype	FMTC	MEN2A	MEN2B
MTC aggressiveness	High	Higher	Highest
Age of onset	Adults	5 years	First year of life
Timing of prophylactic thyroidectomy	When calcitonin rises/5 to 10 years of age	Age 5 years	First months of life
Other endocrine tumours	Rarely	PHEO, HPT	PHEO

PHEO: pheochromocytoma. HRT: hyperparathyroidism.

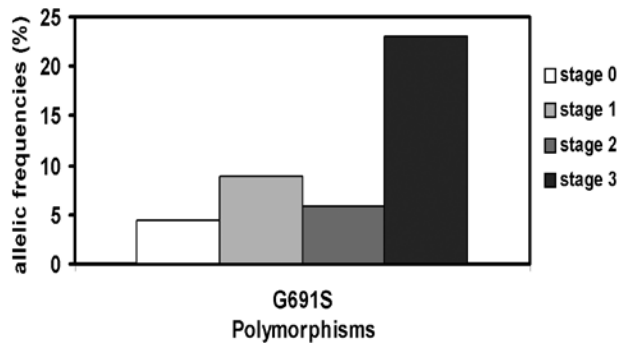


Figure 4. Allelic frequencies of G691S and tumour stage at diagnosis in 56 patients with MEN2.

rare mutations in order to refine the codon specific treatment

Advances in our understanding of the molecular pathways underlying the different phenotypes of MEN2 may help to tailor more individualized therapeutic modalities based on codon-specific inhibition of tumour growth.

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