Virchows Arch (1997) 431:1–9

REVIEW ARTICLE

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The *RET* proto-oncogene in medullary and papillary thyroid carcinoma

Molecular features, pathophysiology and clinical implications

Received: 9 January 1997 / Accepted: 17 February 1997

Abstract The evolution of cancer is a multistep phenomenon, and multiple cellular genetic lesions are involved in the emergence of the malignant neoplasm. Several early events have been implicated in the neoplastic transformation of thyrocytes, and recent reports have described the involvement of specific genetic alterations in different types of thyroid neoplasms: *ras* point mutations are frequently observed in tumours with follicular histology, *gsp* – the mutated form of the alpha subunit of the Gs-protein – is encountered in up to 73% of papillary or follicular thyroid carcinomas, and a high prevalence of *p53* point mutations has been found in anaplastic thyroid carcinomas but not in differentiated follicular tumours. More recent studies revealed that the *RET* proto-oncogene is involved in the oncogenesis of medullary thyroid carcinoma (MTC) and papillary thyroid carcinoma (PTC) by activation of its tyrosine kinase either by point mutation or rearrangement. In this review the most important recently published data on alterations of the *RET* proto-oncogene in heritable and sporadic MTCs and in PTCs will be summarized. Emphasis will be directed to the pathophysiological mechanisms of tumour initiation, the indications and limitations of DNA testing, and the clinical implications of identified *RET* defects in thyroid lesions.

Key words Thyroid carcinoma · *RET* proto-oncogene · Screening

The *RET* proto-oncogene

The *RET* proto-oncogene (*RE*aranged during *T*ransfection) is located on chromosome 10q11.2, has 21 exons and encodes a transmembrane receptor with cytoplasmic tyrosine kinase activity [76]. It is composed of a cytoplasmic tyrosine kinase domain, a short transmembrane domain and a large extracellular domain with a number of highly conserved Ca^{2+}-binding Cys residues near the transmembrane region and a cadherin-like ligand-binding site. *RET* transcripts and protein are expressed in cells and neoplasms of neuroendocrine differentiation, including parafollicular C-cells and medullary thyroid carcinomas (MTCs) [70, 81], adrenal medulla and phaeochromocytomas [71, 88], neuroblastomas [43], parathyroid parenchymal cell precursors [80], and peripheral nerves and their tumours [77].

The *RET* protein is a functional receptor for the glial-cell-line-derived neurotrophic factor (GDNF), a distant member of the transforming growth factor (TGF) beta superfamily [19]. Physiological responses to GDNF require the presence of a novel glycosyl-phosphatidylinositol-linked protein (designated GDNFR alpha) that is expressed on GDNF-responsive cells and binds GDNF with a high affinity. GDNF promotes the formation of a physical complex between GDNFR alpha and *RET*, thereby inducing its tyrosine phosphorylation. Thus, GDNF uses a multi-subunit receptor system in which GDNFR-alpha and *RET* function as the ligand-binding and signalling components, respectively [51, 98, 99].

RET in medullary thyroid carcinoma

MTCs comprise 5–10% of all thyroid carcinomas. The majority of these tumours occur sporadically, but about 20% have a familial background [75]. MTCs are assumed to evolve from neural crest-derived C-cells and are regarded as being closely related to tumours of the disseminated neuroendocrine system. Familial forms of MTC are preceded by bilateral, multicentric C-cell hyperplasia (CCH) which is defined as the presence of at least three low-power magnification (×100) microscopic fields containing more than 50 C-cells and at least 40 cells per cm^2. Residual CCH adjacent to MTC is considered a useful characteristic of familial forms that can aid in distinguishing them from sporadic forms; however, up

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to 25% of familial MTCs do not show accompanying CCH, and reactive or secondary CCH has also been found adjacent to tumours of follicular cell origin and in various non-neoplastic thyroid lesions, as well as in elderly individuals [38, 59].

RET in hereditary MTCs

Several groups have demonstrated that distinct germline mutations in the *RET* proto-oncogene are associated with the dominantly inherited cancer syndromes multiple endocrine neoplasia types 2A and 2B (MEN 2A and MEN 2B) and familial medullary thyroid carcinoma (FMTC). All three of these syndromes share MTCs as part of the disease phenotype [17]. The MEN 2A subtype, which accounts for more than 90% of all cases of MEN 2, is characterized by the additional occurrence of phaeochromocytoma and hyperparathyroidism, resulting from parathyroid hyperplasia or adenoma. Activating mutations in the cysteine-rich extracellular region cause enhanced dimerization of the *RET* tyrosine kinase receptor and autophosphorylation and are causative for MEN 2A and FMTC. Missense germline mutations in one of six codons for Cys in *RET* exons 10 (609, 611, 618 and 620) and 11 (630 and 634) have been identified in 97% of MEN 2A and 87% of FMTC families [26]. In FMTC families MTC is the only clinical feature and the course of the disease is more benign than that of MEN 2A or MEN 2B. In a few FMTC families additional germline mutations have been identified at codons 768 and 804 in *RET* exons 13 and 14, respectively [7, 25, 29]. The effects of these mutations are unclear. The former may alter kinase activity by changing the substrate specificity or the ATP-binding capacity of the receptor [25], and the latter may activate the receptor by altering its interactions with normal cellular substrates or modifying the range of substrates the receptor can phosphorylate. In MEN 2B, which accounts for approximately 5% of all patients with MEN 2, an activating mutation of the tyrosine kinase core domain has been identified in 94% of patients [15, 42, 72]. This mutation at codon 918 in exon 16 replaces methionine with threonine and causes increased autophosphorylation and alteration of the substrate specificity of the tyrosine kinase. The MEN 2B subtype (formerly called mucosal neuroma or Wagenmann-Froboese syndrome) is characterized by MTC and phaeochromocytoma, rare involvement of parathyroids, myelinated corneal nerves, gastrointestinal ganglioneuromatosis and a variety of skeletal abnormalities, such as a marfanoid habitus, pes cavus, talipes equinovarus, slipped capital femoral epiphysis, kyphosis, scoliosis and increased joint laxity [17]. The thyroid tumours tend to occur at an earlier age and pursue a more aggressive course than in patients with MEN 2A and FMTC. The germline mutation in MEN 2B frequently represent new mutations (approximately 50%), and recent studies indicate that de novo mutations nearly always arise on the paternally derived chromosome [14, 56].

Several recent studies have dealt with the transforming activity of the MEN 2A- and MEN 2B-associated *RET* mutations in transfected cell lines [4, 10, 83, 89, 93, 107]. Xing et al. demonstrated that NIH/3T3 stable transfectants expressing *RET* with a MEN 2A- or MEN 2B-type mutation gained a transformed morphology, formed colonies in soft agar and tumours in nude mice. These results confirmed that both MEN 2A- and MEN 2B-type *RET* mutations exert dominant transforming activities in NIH/3T3 cells [107], and Landsvater et al. recently provided evidence that this is also the case in vivo [65]. In other studies it had been shown that MEN 2A mutations induce ligand-independent dimerization of the *RET* protein on the cell surface, leading to activation of its intrinsic tyrosine kinase and that transport of the *RET* protein to the plasma membrane is required for its transforming activity [4]. Further, Pandit et al. demonstrated that the MEN 2B point mutation alters the substrate specificity of receptor tyrosine kinases and that the enhanced oncogenesis associated with the MEN 2B mutation may be due in part to alterations in receptor regulation [83]. Functional analyses revealed that the mutated *RET* proteins were constitutively phosphorylated on tyrosine and that their in vitro kinase activity was significantly higher than that of the wild-type protein [10]. Most recently it has been demonstrated that the tyrosine residue 1062 of the mutated *RET* proteins is essential for the transforming activity of both types of mutations [5] and that different additional tyrosine residues may be involved for MEN 2A (tyrosine 905) and MEN 2B (tyrosine 864, 952) [46].

Although several groups have shown that a single *RET* mutation is sufficient to activate and transform *RET* in vitro, there are huge differences in the age of MTC onset among members of MEN 2 families, and the clinical penetrance of the MEN 2 syndrome is not 100%. Therefore it is reasonable to postulate that a germline *RET* mutation may be sufficient to induce CCH but that secondary events are required for the development of MTCs. Thus, several somatic defects, such as loss of parts of chromosomes 1p, 3p and 22q and somatic *RET* mutations, have been described in MTCs of MEN 2 patients [68, 73]. However, further studies on larger series of tumours from MEN 2 patients are necessary to substantiate this hypothesis.

In families with MEN 2, genetic analysis of the *RET* proto-oncogene can be used to confirm the diagnosis and to identify asymptomatic family members with the syndrome. Furthermore, to a certain extent, it allows the prediction of a particular phenotype in a disease gene carrier. Thus, studies on the correlation between genotype and disease phenotype in 477 MEN 2 families revealed that disease gene carriers with codon 634 mutations more frequently develop phaeochromocytoma and that a specific mutation at codon 634, changing Cys to Arg, is associated with hyperparathyroidism [26]. However, the latter correlation has been questioned by other groups [30, 91].

Molecular screening for *RET* mutations is most easily performed by PCR-based methods using single-strand

conformation polymorphism (SSCP), heteroduplex or denaturing gel electrophoresis, followed by direct sequencing or restriction endonuclease digestion of PCR products [6, 52, 60, 67]. Furthermore, we and others have demonstrated that DNA testing can also be performed on DNA extracted from paraffin-embedded tissues, allowing retrospective analyses [58, 61].

First-degree family members of MEN 2A or FMTC gene carriers should be tested for *RET* mutations before the age of 5 years [102], since measurement of calcitonin levels after stimulation with pentagastrin can yield false-positive and false-negative results [66, 102]. In MEN 2B families DNA testing should be performed as soon as possible, since MTC may develop at a younger age than in the MEN 2A and FMTC subtypes. Disease gene carriers should be tested for MTC, phaeochromocytoma and hyperparathyroidism on a regular basis and offered prophylactic thyroidectomy [102]. The appropriate age for prophylactic thyroidectomy, however, is still debated. Hence, the penetrance of MEN 2 is approximately 70% at 70 years of age, but more than 95% of all disease gene carriers will have positive pentagastrin stimulation tests (due to CCH or MTC) by the age of 35 [22]. Prophylactic thyroidectomy in childhood appears to be the therapy of choice for asymptomatic disease gene carriers. Most specialists recommend surgery before the age of 5 in MEN 2A gene carriers and as soon as possible in children with MEN 2B [35, 64, 81, 85], since metastatic MTC may also arise in children as young as 3 and 5 years of age in MEN 2B and MEN 2A gene carriers, respectively [35, 53]. Prophylactic thyroidectomy appears to be associated with minimal morbidity and virtually no mortality [64]. Furthermore, autotransplantation of parathyroids is not required and lymph nodes of the central compartment need not be dissected in prophylactic thyroidectomy, in contrast to therapeutic thyroidectomy in patients with established MTC or positive pentagastrin stimulation tests [62]. Moreover, a significant number of patients who undergo thyroidectomy after the diagnosis of MTC have persistent or recurrent disease in the long term [92]. Total parathyroidectomy together with heterotopic autotransplantation, however, is advocated in gene carriers of MEN 2A families with hyperparathyroidism, since recurrent or persistent hyperthyroidism frequently occurs after selective or subtotal parathyroidectomy, as a result of either missed glands or interval development of neoplasia in previously normal parathyroid glands left in situ [39].

RET in sporadic MTCs

Somatic mutations in *RET* have also been found in a proportion of patients with sporadic MTCs. By far the most common mutation involves codon 918 (Met→Thr). This type of missense mutation in exon 16 has been described in 23–85% of sporadic MTCs and in our own series of 16 tumours we found 44% MTCs harbouring this type of *RET* mutation. Other groups found additional mutations at codon 768 (Glu→Asp) of exon 13 [25, 72], at codon 883 (Ala→Ser) of exon 15 [24], codon 634 of exon 11 [54, 86] and exon 10 [24] in a small proportion of tumours. Furthermore, microdeletions causing the loss of a Cys residue at codon 630 or 634 have been described in sporadic MTCs by several groups [1, 18, 49, 54]. The differences in mutational frequencies and codons involved that have been reported by various centres suggest that either regional and environmental or technical factors might be involved. Thus, in a recent study Eng et al. examined microdissected subpopulations from sporadic MTCs and multiple metastases from these tumours and found that approximately 80% of sporadic MTCs had at least one subpopulation with the *RET* codon 918 mutation [27]. The distribution of this mutation was non-homogeneous, occurring only in subpopulations in most tumours and among subsets of multiple metastases, thus implying that although the codon 918 mutation could be an early event, it is not necessarily an early or essential event in oncogenesis. These findings suggest either that the codon 918 mutation can arise as an event in progression within a metastatic clone or within a single tumour, or that MTC can be of polyclonal origin. In the same study the authors also reported that one of two MTCs from MEN 2A patients carried a somatic mutation at codon 918 in addition to the *RET* mutation present in the germline.

Romei et al. and Zedenius et al. recently demonstrated that somatic mutations of the *RET* proto-oncogene in sporadic medullary thyroid carcinoma were significantly correlated with a poor outcome with regard to distant metastasis or tumour recurrence [88, 108]. Marsh et al., however, in a study on 32 patients, reported that the presence or absence of the somatic mutation at the *RET* codon 918 was not correlated with age at diagnosis, tumour size, presence or absence of metastases, MTC-related morbidity, or baseline calcitonin levels at diagnosis or most recent follow-up [69]. Thus, the clinical significance of *RET* mutations in sporadic MTCs remains to be determined in larger series of patients with longer periods of follow-up.

We and others have demonstrated that the analysis of germline DNA for *RET* mutations may be helpful to determine the hereditary or sporadic nature of MTCs [29, 60] and that DNA analysis can also be performed on DNA extracted from paraffin-embedded tissues [58, 61]. In patients with hereditary MTCs, the specific *RET* mutation will be present both in tumour DNA and in all normal tissues and blood cells harbouring constitutional (germline) DNA. The absence of a germline *RET* exon 10, 11, 13, 14 or 16 mutation appears to rule out MEN 2A, 2B or FMTC with a high probability, although a familial form of MTC other than classical MEN 2 cannot be conclusively excluded [23]. Thus, the presence of a few MEN 2 families without detectable *RET* mutations [97] indicates that hereditary MTCs might also be caused by germline mutations in the gene encoding the GDNF receptor alpha or other genes. Rates of de novo mutations in MEN 2A and FMTC appear to be approximately

6% and those of MEN 2B, around 50% [23, 106]. The best policy for evaluating apparently sporadic cases of MTC and phaeochromocytoma is still debated. We and others recommend routine application of *RET* proto-oncogene testing in all patients with apparently sporadic MTCs and phaeochromocytomas [106], whereas others prefer a more conservative and cost-effective approach with the histopathological features of surgical specimens and the patient's age at presentation taken into consideration. Only if the patient is less than 40 years old or has CCH together with MTC or multifocal tumours should DNA testing be performed [22]. Since a subset of phaeochromocytomas may be associated with von Hippel-Lindau disease, patients with familial, multiple, or early-onset phaeochromocytoma should not only be investigated for *RET* but also for germline VHL gene mutations [16].

Some MEN 2A families present with skin amyloidosis, and Hofstra et al. recently demonstrated that individuals with autosomal dominant familial cutaneous lichen amyloidosis, which is more generalized, in their pedigrees do not carry *RET* mutations. They concluded that the dermal amyloidosis found in some MEN 2A families and in familial cutaneous lichen amyloidosis are different conditions [40]. Consequently, patients with apparently familial cutaneous lichen amyloidosis do not appear to be at risk for MEN 2A and need not be screened for RET mutations.

RET in papillary thyroid carcinoma

The *RET* proto-oncogene has also been implicated in the causation of PTCs, which compose approximately 80% of all thyroid carcinomas [75], especially in iodine-rich areas, indicating that stimulation of follicular cells by elevated TSH is of lesser importance in the oncogenesis of PTCs than of follicular thyroid carcinomas. Several studies have demonstrated that *RET* is activated through somatic rearrangements in a subset of PTCs. The *RET*/PTC oncogenes are rearranged forms of the *RET* proto-oncogene and encode fusion proteins in which proto-*RET* tyrosine kinase and C-terminal domains are fused to different donor genes. The respective *RET*/PTC oncoproteins display constitutive tyrosine kinase activity and tyrosine phosphorylation. Three major forms of the *RET*/PTC oncogene have been identified; the *RET*/PTC-1 oncogene (where c-*RET* rearranges with the H4 gene D10S170 on chromosome 10q21), the *RET*/PTC-2 oncogene (where c-*RET* rearranges with the regulatory subunit R1 alpha of the protein kinase A on 17q23) and the *RET*/PTC-3 oncogene (where c-*RET* rearranges with the *RFG2*/*Ele1* gene on 10q11.2) [8, 94, 96]. Thus, the two most common forms, *RET*/PTC-1 and *RET*/PTC-3, both result from a paracentric inversion of the long arm of chromosome 10. More recently, a novel type of *Ele1*/*RET* rearrangement designated *RET*/PTC-4 has been described, where the exon 5 of *Ele1* is joined to exon 11 instead of exon 12 of *RET* and the cDNA sequence is 93 nucleotides larger than the regular one [32]. The *RET*/PTC-4 oncogene has been found in a post-Chernobyl PTC, indicating that targeted radiation effects could be responsible for the atypical *RET* rearrangement.

Identification of *RET* proto-oncogene activation in PTCs has been achieved by several different methods. These include Southern blotting, requiring high-quality DNA extracted from unfixed tumour and nontumour tissues [31] and reverse transcription (RT)-PCR-based techniques to identify both specific *RET* rearrangements [95] and gene activation [104]. The latter methods are also suitable for the analysis of archival materials, and Williams et al. have demonstrated that the use of a nested RT-PCR approach for the analysis of *RET* tyrosine kinase expression can significantly improve the sensitivity of their assay [104]. A third method of analysing *RET* overexpression in PTCs is the immunohistochemical detection of the *RET* gene product in tissue sections [100]. In our own experience, however, the currently commercially available *RET* antibodies usually yield high background staining and the results are therefore difficult to interpret.

Wide differences (2.5–60%) in frequency of *RET* activation by *RET*/PTC in PTCs of different populations have been reported, and it is not clear whether these are due to environmental factors, racial differences or technical reasons [31, 37, 78, 101, 104, 109]. However, several studies have shown an association between ionizing radiation and development of PTC [31, 45, 57]. In addition, in vitro irradiation of tumour cell lines induced rearrangements of *RET* similar to those observed in human PTCs [44]. These two observations could be related to the reported increased incidence of PTCs in children living in contaminated areas around Chernobyl, given that it has been demonstrated that about 60% of them present a *RET* oncogenic activation. However, Williams et al. recently identified a similar proportion of *RET*/PTC-1-positive PTCs in nonirradiated patients [105], and Bongarzone et al. demonstrated that age per se also plays a part in the development of *RET*-positive PTCs. The latter authors found in a series of 92 consecutive patients that the frequency of *RET* activation is significantly higher in the group of patients aged 4–30 years, supporting the concept that age could be contributing to this thyroid-specific carcinogenic process [9]. Furthermore, Viglietto et al. recently showed, by using a combined immunohistochemical and RT-PCR-based approach, that *RET*/PTC activation is present in 11 out of 26 (42%) occult PTCs and concluded that *RET*/PTC rearrangement represents an early event in the process of thyroid cell transformation [100].

Recently, two groups demonstrated that transgenic mice with thyroid-targeted expression of the *RET*/PTC-1 oncogene developed thyroid carcinomas with considerable similarities to human PTCs, particularly in the nuclear cytological features and the presence of local invasion [50, 90]. Furthermore, it has been shown that Tyr-539 of *RET*/PTC-2 (Tyr-761 on the proto-*RET* product) is an essential docking site for the full transforming potential of the oncogene. In addition, PLC-gamma has

been identified as a downstream effector of *RET*/PTCs, and it has been suggested that this transducing molecule could play a crucial role in neoplastic signalling triggered by *RET*/PTC oncoproteins [11]. Other studies have shown that only the dimerization domain of R1 alpha fused to *RET* is required and that two tyrosines outside the conserved kinase core are also essential for full mitogenic activity of *RET*/PTC-2. These two tyrosines, Tyr-350 and Tyr-586, are potential sites for Src homology 2 and phosphotyrosine-binding domain interactions [20].

The clinical relevance of *RET*/PTC rearrangements in PTCs is still controversial. Some have suggested that *RET*/PTC expression could serve as an indicator of aggressive behaviour in PTCs, specifically for distant metastatic disease [47, 48]. In a recent study Sugg et al. analysed 60 thyroid carcinomas by RT-PCR for *RET*/PTC expression to determine a possible correlation with clinical history, disease stage, or tumour morphology. *RET*/PTC oncogene rearrangements were found in 3 of 60 (5%) thyroid carcinomas. One papillary carcinoma (1.7%) was positive for *RET*/PTC-1, none for *RET*/PTC-2, and 2 (3.4%) were positive for *RET*/PTC-3. Although *RET*/PTC oncogene rearrangements were not present in tumours with aggressive morphological features, they found *RET*/PTC rearrangements in young patients (<45 years of age) with small thyroid carcinomas showing a predisposition for lymphatic involvement, suggesting a possible role in the development of this subgroup of tumours [95]. Thus, more data are necessary to determine whether molecular analysis of *RET*/PTC rearrangements are required in patients with PTCs.

The *RET*/PTC rearrangements are undoubtedly crucial to the understanding of PTC oncogenesis and may be especially important in radiation-induced thyroid cancers; however, a substantial proportion of PTCs do not show *RET*/PTC rearrangements. Those tumours are probably caused by other genetic alterations, including rearrangements of the *TRK* tyrosine kinase [101] or other, still unidentified, genes. Furthermore, the activation of *RET* by other mechanisms or rearrangements that are currently not yet identified might also be involved in the carcinogenesis of PTCs. These might explain the slightly higher proportion of PTCs with *RET* activation identified by Williams et al. using an RT-PCR approach to identify *RET* mRNA expression compared with results obtained from studies identifying specific types of *RET* rearrangements with Southern blotting or RT-PCR [104].

RET in other sporadic neuroendocrine tumours and Hirschsprung's disease

In an attempt to investigate the role of the *RET* proto-oncogene in sporadic neuroendocrine tumours, we examined a series of 112 tumours for the presence of *RET* mutations. In none of the investigated neuroendocrine tumours of the pancreas, gastrointestinal tract, bronchopulmonary system, pituitary, adrenal medulla or skin has a mutation in *RET* exons 10, 11, 13, 15 or 16 been found [63]. These findings have been challenged by a Japanese group who described somatic mutations in codon 664 of *RET* in a few small cell cancers [33, 34]. However, the absence of *RET* mutations in neuroblastomas has recently been confirmed by Hofstra et al., who performed an SSCP analysis of all *RET* exons on 16 sporadic and familial tumours. From this study the authors concluded that the high expression of *RET* in neuroblastoma most probably reflects the differentiation status of the tumour cells and that *RET* does not have a crucial role in oncogenesis in neuroblastoma [41]. In a further study we have found none of the previously described MEN 2A or 2B mutations in the DNA of 33 sporadic hyperplastic and neoplastic lesions of the parathyroid gland [82], a finding which has recently been confirmed by others [55, 84, 103]. Collectively, these results indicate that *RET* might not be generally important in the formation of sporadic neuroendocrine tumours other than MTC and phaeochromocytoma.

A variety of inactivating mutations of the *RET* proto-oncogene, which result in defective protein formation, as well as mutations in GDNF, have been found in 10–40% of patients with Hirschsprung's disease, a congenital disorder characterized by absent enteric innervation [3, 13, 21, 79]. It occurs in 1 in 5000 live births and can be sporadic or familial. When it is familial, it can be autosomal dominant or recessive and its penetrance is low (approximately 30%). In contrast to the *RET* mutations in MEN 2, the mutations in Hirschsprung's disease are scattered along the length of the gene and lead to an inactivation of the receptor by truncation or missense mutation. Interestingly, the combination of MEN 2 and Hirschsprung's disease has been described in several families [2, 12, 74]. All these families had *RET* mutations in codon 618 or 620 and different degrees of penetrance in various tissues and the existence of modifier loci have been invoked to explain the cosegregation of MEN 2 and Hirschsprung's disease in these kindreds.

From a clinical point of view it is not practical to offer routine testing for *RET* mutations in all patients with Hirschsprung's disease because the penetrance of the gene is low and the mutation causing the disease can occur in any of the 21 exons of *RET* [22].

Summary and conclusions

The *RET* proto-oncogene has not only been conclusively identified as responsible for the three subtypes of the inherited cancer syndrome MEN 2, but also shown to be involved in the molecular evolution of sporadic MTCs and PTCs and of Hirschsprung's disease. A variety of recent studies have elucidated the pathophysiological mechanisms leading to neoplastic disease, and we now understand that dominant activating germline mutations lead to MEN 2A, MEN 2B and FMTC, somatic mutations to sporadic MTCs, *RET* rearrangements to PTCs and inactivating alterations to Hirschsprung's disease. The clinical significance of *RET* alterations, especially in

sporadic thyroid tumours, is still controversial. Therapeutic concepts in MEN 2 gene carriers are only starting to emerge. We are now challenged by the task of collecting more clinical, biochemical and molecular data from patients with hereditary and sporadic MTCs and PTCs in order to evaluate the clinical importance of identified *RET* abnormalities in particular patients. Furthermore, we should centralize the data on MEN 2 families and patients with thyroid carcinoma in order to achieve an international interdisciplinary consensus on the diagnostic and therapeutic approaches to be adopted in disease gene carriers. In this way we will improve our understanding of the influence of environmental and racial factors in oncogenesis in sporadic MTCs and PTCs.

Acknowledgements I am grateful to P. U. Heitz and J. Roth for general support, P. Saremaslani and S. Muletta-Feurer for excellent technical assistance and C. Eng for helpful discussions.

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