0021-972X/01/$03.00/0
The Journal of Clinical Endocrinology & Metabolism
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Vol. 86, No. 3
Printed in U.S.A.

Genotype-Phenotype Correlations in Hereditary Medullary Thyroid Carcinoma: Oncological Features and Biochemical Properties*

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ABSTRACT

In hereditary medullary thyroid carcinoma (MTC), few genotype-phenotype correlations have been established. RET genotypes (exons 10, 11, 13, and 14) of 63 patients with hereditary MTC (from November 1994 to October 1999) were correlated with age at diagnosis, sex, the TNM system, and basal calcitonin levels. Mutations in exons 10, 11, 13, and 14 were demonstrated in 22% (14 of 63), 54% (34 of 63), 21% (13 of 63), and 3% (2 of 63). The median ages at diagnosis differed significantly (38, 27, 52, and 62 yr; $P = 0.003$). When grouped by cysteine codons (exons 10 and 11 *vs.* exons 13 and 14), this difference became even more evident (30 *vs.* 56 yr; $P = 0.001$). Apart from age at diagnosis, no other significant associations were noted. Based hereon, three MTC risk groups were devised according to genotype: a high risk group (codons 634 and 618) with the youngest ages of 3 and 7 yr at diagnosis; an intermediate risk group (codons 790, 620, and 611) with ages of 12, 34, and 42 yr; and a low risk group (codons 768 and 804) with ages of 47 and 60 yr, respectively. Age at diagnosis was unrelated to specific nucleotide and amino acid exchange within each codon.

The current data demonstrate that there is a significant genotype-phenotype correlation, allowing for a more individualized approach to the timing and extent of prophylactic surgery. (*J Clin Endocrinol Metab* **86:** 1104–1109, 2001)

MEDULLARY THYROID carcinoma (MTC), a neoplasm of parafollicular C cell origin, may be sporadic or, less frequently, may occur on a hereditary basis. In the presence of pheochromocytoma or hyperparathyroidism, hereditary MTC forms part of an autosomal, dominantly inherited cancer syndrome designated multiple endocrine neoplasia (MEN) type IIa, which accounts for 90% of MEN II cases (1, 2). In MEN IIb, an extremely aggressive variant of MTC appears in conjunction with a marfanoid habitus, ganglioneuromatosis, and bumpy lips (2, 3). More recently, evidence has emerged suggesting a separate variant of familiar MTC (FMTC) that occurs in isolation (4). Traditionally, biochemical screening for elevated basal and stimulated serum calcitonin levels has been used to identify those patients at risk to develop hereditary MTC. Unfortunately, false test results have occurred with biochemical screening, resulting in some gene carriers being missed.

In 1993, the genetic background of hereditary MTC has been elucidated (5, 6). Genetic testing for germline mutations in the RET protooncogene has become available and today forms the basis for DNA-based screening procedures. Molecular biology now affords an early identification of carriers of RET protooncogene germline mutations who are bound to develop MTC later in life. In these patients, early prophylactic thyroidectomy must be envisaged to ensure definitive cure. However, no universal consensus exists as to the optimal timing and extent of prophylactic surgery in these patients. Some investigators (1, 7–9) have advocated 2, 5, and 6 yr of age, respectively, for prophylactic surgery, with the objective of removing the thyroid gland before malignant progression from C cell dysplasia to medullary carcinoma has occurred. This strategy would also eliminate the need for additional lymph node dissection, which results in increased morbidity (10). Once lymphatic dissemination has occurred, biochemical cure, as defined by a postoperative normalization of serum calcitonin, may be beyond reach despite radical surgery on the neck and mediastinum.

With the advent of molecular testing for germline mutations, the time may have come to classify hereditary MTC on the basis of genotype rather than adhering to phenotyping by the use of calcitonin and pentagastrin stimulation tests. Genotyping also holds the prospect of replacing the traditional categories of MEN IIa and FMTC, which may be outdated (11). The current study was devised to investigate the impact of distinct germline mutations on oncological features of hereditary MTC, thus providing a basis on which individual recommendations for the optimal timing of prophylactic surgery can be made. Timely prophylactic surgery would help avoid additional central lymph node dissection and thus decrease surgical morbidity (10).

Received May 1, 2000. Revision received October 31, 2000. Accepted November 6, 2000.

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* This work was based on a previous communication to the Annual Meeting of the British Association of Endocrine Surgeons, Edinburgh, United Kingdom, May 27–28, 1999.

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Subjects and Methods

Patient selection

Of 198 patients operated on for MTC between November 1994 and October 1999 at this institution, 63 patients (32%) had hereditary MTC with confirmed germline mutations in exons 10, 11, 13, and 14 and thus qualified for enrollment in this study. Before undergoing genetic testing, all patients and their legal guardians, respectively, had given informed consent in accordance with institutional guidelines and national regulation. To create a more homogeneous study population, carriers of codon 918 germline mutations (3.5%; 7 of 198) were not considered for the series because of their well known distinct phenotype, the characteristic onset of MTC in early infancy, and the frequent coincidence of lymphatic and distant metastases by the time of diagnosis. All patients received at least a standard total thyroidectomy in conjunction with a standard systematic cervico-central lymph node dissection, as described previously (12). For determination of preoperative serum calcitonin levels, a highly sensitive immunoradiometric assay (CIS-Bio International, Gif-sur-Yvette, France) was employed, with a normal range of less than 10 pg/mL. Molecular data (germline mutations in exons 10, 11, 13, and 14 and codons 611, 618, 620, 634, 768, 790, and 804, respectively) were then correlated with demographic data (patient age at diagnosis of MTC and patient sex), histopathological features (pT, pN, and M categories and UICC stage), and biochemical properties (basal calcitonin levels) from the patients' charts. In each genotype group, particular attention was devoted to the youngest MTC patient without (pN0) or with (pN1) lymph node metastases and without biochemical cure.

Pathological examination and tumor staging

For histopathological analysis, the thyroid gland was divided vertically to separate the left and right lobes, and the two halves were then sliced horizontally as described previously (13). After fixation in formalin, the entire specimen was embedded in paraffin. Soft tissue adjacent to lymph nodes was processed separately. Both conventional staining (hematoxylin and eosin) and calcitonin immunohistochemistry were performed on every surgical specimen, using the standard avidin-biotin complex peroxidase approach and a commercial polyclonal antibody (Immunotech, Marseilles, France). The diagnosis of MTC was made when there was evidence of extension beyond the basement membrane, demonstration of lymphatic or vascular invasion on histopathology, or a combination of both. Tumor staging was performed according to the current UICC TNM classification (14).

DNA preparation and PCR amplification

For identification of RET germline mutations, genomic DNA was purified from peripheral blood lymphocytes using the QIAMP blood kit (QIAGEN, Hilden, Germany). Genomic DNA was amplified using PCR and the following oligonucleotide primers for exons 10, 11, 13, and 14, as described previously (15): exon 10, Ret10F (5′-GCAGCATTGTT-GGGGGACA-3′) and Ret 10R (5′-GTCCCGGCCACCCACT-3′; size of amplified fragment, 140 bp); exon 11, Ret 11F (5′-CATGAGGCAGAG-CATACGCA-3′) and RET11R (5′-GACAGCAGCACCGAGACGAT-3′; size of amplified fragment, 156 bp); exon 13, Ret13F (5′AACTT-GGGCAAGGCCATCA-3′) and Ret13R (5′-AGAACAGGGCTGTAT-GGAGC-3′; size of amplified fragment, 108 bp); and exon 14, Ret14F (5′-AAGACCCAAGCTGCCTGAC-3′) and Ret14R (5′-GCTGGGTGCA-GAGCCATAT-3′; size of amplified fragment, 294 bp). One hundred nanograms of DNA were amplified in a Perkin-Elmer Corp. 9600 thermocycler (Palo Alto, CA) in a volume of 25 μL containing 1 μmol/L of each oligonucleotide primer, 10 mmol/L Tris-HCl (pH 8.3), 2.5 mmol/L $MgCl_2$, and 1 U *Taq* polymerase (Roche, Branchburg, NJ). The PCR was started with 1 min of denaturation at 95 C, followed by 35 cycles of 1 min each at 65, 72, and 95 C, and was completed with 5 min at 72 min. The amplified DNA was analyzed on a 2% agarose gel and purified with the QIAGEN Quickspin kit.

Single strand conformational polymorphism analysis and direct sequencing

For single strand conformational polymorphism analysis of exons 10, 11, 13, and 14, the amplified DNA fragments were denatured in formamide-50 μmol/L ethylenediamine tetraacetate and cooled on ice before loading onto the gel. Separation was carried out in a vertical gel electrophoresis apparatus in an MDE gel (AT Biochem, Malvern, PA) at 4 C (exon 10), and in a 12% polyacrylamide-0.8% bis-acrylamide gel at 45 C (exon 11), 30 C (exon 13), or room temperature (exon 14) at 240–300 mV for 10–16 h. DNA bands were visualized by silver staining according to standard procedures. PCR-amplified DNA was sequenced by direct cycle sequencing using the fluorescent-labeled dideoxy terminators (dRhodamine Terminator Cycle Sequencing Ready Reaction Kit, PE Applied Biosystems, Foster City, CA) and run on the automated sequencer 377 from PE Applied Biosystems.

Statistical analysis

Associations between categorical and metric parameters were tested using two-tailed Fisher's exact test and the Mann-Whitney-Wilcoxon and Kruskal-Wallis rank sum tests, respectively. To assess correlations between metric parameters, Spearman's correlation coefficient was calculated. The level of significance was set at 0.05.

Results

Patient age at diagnosis by exon mutation

With 54% of cases, germline mutations in codon 634 of exon 11 dominated the series (Tables 1 and 2), followed by mutations in codons 618 and 790 (14% each), codon 768 (6%), codon 611 (5%), and codons 620 and 804 (3% each). With respect to patient age at diagnosis, the hereditary MTC differed significantly (Table 1) when broken down by mutated exon (median ages of 38, 27, 52, and 62 yr in exons 10, 11, 13, and 14; $P = 0.003$; $r = 0.24$). When the mutated exons were grouped by cysteine (exons 10 and 11) and noncysteine codons (exons 13 and 14), this difference (median ages of 30 *vs.* 56 yr; $P = 0.001$; $r = 0.42$) was even more pronounced. A similar rank order (7, 3, 12, and 60 yr in exons 10, 11, 13, and 14, respectively) was found when the youngest age at diagnosis was regarded subsequently.

Patient age at diagnosis by codon mutation

Broken down by mutated codon in a subsequent analysis (Table 2), the differences in age presentation remained significant (median ages of 44, 29, 36, 27, 60, 39, and 62 in codons 611, 618, 620, 634, 768, 790, and 804; $P = 0.009$; $r = 0.22$). Codons predisposing to younger age at diagnosis (codons 634 and 618) tended to be more common (54% and 14%) than those (codons 768 and 804) associated with a high age at diagnosis (6% and 3% each). When grouped by their respective genotypes, MTC patients markedly differed in their youngest age at diagnosis regardless of whether they had lymph node metastases. These appreciable differences in age presentation allowed us to establish a hierarchy in codon mutations and to devise risk categories for the development of MTC according to mutated codon: a high risk group (codons 634 and 618) with youngest ages of 3 and 7 yr at diagnosis; an intermediate risk group (codons 790, 620, and 611) with youngest ages of 12, 34, and 42 yr; and a low risk group (codons 768 and 804) with youngest ages of 47 and 60, respectively. The youngest patient with MTC and additional lymph node metastases, who was 9 yr old and not biochemically cured, revealed a $Cys^{634}Arg$ germline mutation. As illustrated in Tables 1 and 2, lymph node metastases frequently precluded biochemical cure.

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TABLE 1. Oncological features and biochemical properties grouped by exon mutation

Exons	10	11	13	14	*P*	Cys[+a]	Cys[−a]	*P*
n (%)	14 (22)	34 (54)	13 (21)	2 (3)		48 (76)	15 (24)	
Index patient (%)	64	50	62	100	0.58	54	67	0.55
Median age at diagnosis, yr (25/75% quartiles)	38 (19–44)	27 (12–41)	52 (31–66)	62 (60–63)	0.003	30 (13–42)	56 (33–63)	0.001
Youngest patient (yr)								
With MTC	7	3	12	60		3	12	
With pN1	21	9	56	60		9	56	
Without biochemical cure	37	9	56	60		9	56	
Sex (% male)	43	56	23	100	0.09	52	33	0.25
pT1 (%)	43	62	77	50		56	73	
pT2 (%)	43	29	8	50		33	13	
pT3 (%)	7	3	15	0		4	13	
pT4 (%)	7	6	0	0	0.31	6	0	0.18
pTXb (%)	50	82	62	50	0.09	72	60	0.52
pN1 (%)	57	50	38	100	0.48	52	47	0.77
pN1b (%)	77	56	40	50	0.51	63	43	0.41
M1 (%)	0	12	15	0	0.50	8	13	0.62
UICC stage 1 (%)	36	44	62	0		42	53	
UICC stage 2 (%)	7	6	0	0		6	0	
UICC stage 3 (%)	57	41	23	100		46	33	
UICC stage 4 (%)	0	9	15	0	0.44	6	13	0.50
Median basal calcitonin, pg/mL (25/75% quartiles)	38 (8–763)	125 (34–490)	170 (5–1484)	1596 (791–2400)	0.36	101 (17–516)	204 (7–1811)	0.67

[a] Mutations in cysteine domains (exons 10 and 11) *vs.* mutations outside cysteine domains (exons 13 and 14).

TABLE 2. Oncological features and biochemical properties grouped by codon mutation

Codon	611	618	620	634	768	790	804	*P*
n (%)	3 (5)	9 (14)	2 (3)	34 (54)	4 (6)	9 (14)	2 (3)	
Index patient (%)	100	44	100	50	50	67	100	0.42
Median age at diagnosis, yrs (25/75% quartiles)	44 (42–45)	29 (14–48)	36 (34–38)	27 (12–41)	60 (48–73)	39 (23–61)	62 (60–63)	0.009
Youngest patients (yr)								
With MTC	42	7	34	3	47	12	60	
pN1	45	21	38	9	68	56	60	
Without biochemical cure	45	37	38	9	68	56	60	
Sex (% male)	33	56	0	56	0	33	100	0.13
pT1 (%)	33	44	50	62	75	78	50	
pT2 (%)	67	33	50	29	0	11	50	
pT3 (%)	0	11	0	3	25	11	0	
pT4 (%)	0	11	0	6	0	0	0	0.61
pTXb (%)	33	56	50	82	100	44	50	0.06
N1 (%)	33	67	50	50	50	33	100	0.72
pN1b (%)	100	71	100	56	50	33	50	0.94
M1%	0	0	0	12	25	11	0	0.79
UICC stage 1 (%)	33	33	50	44	50	67	0	
UICC stage 2 (%)	33	0	0	6	0	0	0	
UICC stage 3 (%)	33	67	50	41	25	22	100	
UICC stage 4 (%)	0	0	0	9	25	11	0	0.66
Median basal calcitonin, pg/mL (25/75% quartiles)	9 (2–518)	45 (16–1500)		125 (34–490)	187 (44–11200)	20 (5–1483)	1596 (791–2400)	0.53

Patient sex, TNM classification, and serum calcitonin by RET mutation

Neither mutated exons nor codons differed significantly with regard to sex distribution. There were no significant differences among the pT, pN, and M categories, UICC tumor stages, and preoperative basal serum calcitonin regardless of whether the figures had been grouped by mutated exon (Table 1) or codon (Table 2).

Oncological features and biochemical properties in index and nonindex patients

Of the 63 patients, 36 were index patients. In these index patients, patient age at diagnosis significantly differed by RET mutation. Grouped by exon ($P = 0.003$; $r = 0.41$), the median ages at diagnosis were 40 yr (exon 10), 30 yr (exon 11), 57 yr (exon 13), and 62 yr (exon 14), respectively. Grouped by codon ($P = 0.019$; $r = 0.38$), the median ages at diagnosis were 44 yr (codon 611), 35 yr (codon 618), 36 yr (codon 620), 30 yr (codon 634), 60 yr (codon 768), 57 yr (codon 790), and 62 yr (codon 804), respectively. RET mutations in cysteine codons (exons 10 and 11) significantly correlated with younger patient age at diagnosis (medians of 33 *vs.* 59 yr; $P < 0.001$; $r = 0.60$) relative to RET mutations in noncysteine codons (exons 13 and 14). In the 27 nonindex patients, there were no significant associations between exon or codon mutations and patient age at diagnosis. Apart from

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patient age at diagnosis, none of the other oncological and biochemical parameters significantly correlated with RET mutations when analyzed separately for index and nonindex patients (data not shown).

Patient age at diagnosis by nucleotide and amino acid exchange

Except for codon 634, all nucleotide and amino acid exchanges within the codons 611, 618, 620, 768, 790, and 804 occurred at more or less comparable rates (Table 3). In codon 634, the Cys^{634}Arg (TGC→CGC) genotype prevailed, accounting for 50% (17 of 34) of affected patients and 52% (11 of 21) of affected families. This finding suggests that the Cys^{634}Arg (TGC→CGC) genotype occurs more frequently than would be expected merely by chance. Age at diagnosis did not seem to differ significantly among the various nucleotide and amino acid exchanges within each codon, indicating a similar degree of RET protooncogene activation.

Discussion

The RET protooncogene

The RET protooncogene is expressed in cells of neuronal and neuroepithelial origin and encodes a receptor tyrosine kinase (3). The first 10.5 exons encode the extracellular region, which includes a cadherin-like and a cysteine-rich domain. The intracellular tyrosine kinase domains and the C-terminal tail are encoded by the remaining exons (16, 17). The highly conserved cysteine domains are pivotal in maintaining the secondary and tertiary structures of the RET extracellular domain (18). Mutations in these cysteine domains, *i.e.* in codons 609, 611, 618, and 620 of exon 10, and of codon 634 of exon 11, enhance the ligand-independent dimerization and cross-phosphorylation, thus allowing constitutive protein tyrosine kinase activity in the absence of the ligand (19). Other mutations in the intracellular region of the RET protooncogene, *i.e.* in codons 768 and 790 of exons 13 and in codon 804 of exon 14, are located in or near a region implicated in ATP binding (20, 21). Mutations in codon 918 of exon 16 directly alter the substrate recognition pocket of the catalytic core within the tyrosine kinase domain, improving its affinity to the ligand. Its hallmark is an early-onset variant of MTC as part of the MEN II syndrome, which is frequently fatal, having already spread to lymph nodes and distant organs by the time of diagnosis (2). As may be inferred from the different mechanisms of receptor activation, the location of individual RET protooncogene mutations on the subcellular level may have a direct impact on the disease phenotype.

RET protooncogene mutations and disease phenotype

Most genotype-phenotype correlations to date have focused on the relationship between specific RET protooncogene genotypes and disease phenotype. Correlating 477 MEN II families from 18 tertiary referral centers, a statistically significant association ($P < 0.001$) was found between the presence of any mutation at codon 634 and the presence of pheochromocytoma and hyperparathyroidism (2). Within the codon 634 mutations, C634R (TGC→CGC; Cys→Arg) mutations, as opposed to any other codon 634 mutation, were significantly ($P = 0.002$) associated with the presence of hyperparathyroidism. Of note, there were no C634R mutations among patients with FMTC (2, 3). Conversely, no mutations at codon 768 (E768D) and codon 804 (V804L) have been observed until very recently in MEN IIA or MEN IIB families (2). Concomitant Hirschsprung's disease, which represents a loss of function mutation in the RET protooncogene, segregated with codons 620 (5 of 6) or 618 (1 of 5) only. In each case, a TGC to CGC mutation was present, resulting in an exchange of asparagine for cysteine (2). Conversely, coexistent cutaneous lichen amyloidosis exclusively (18 of 18) segregated with codon 634 (2).

RET protooncogene mutations and oncological features

A systematic correlation of patient age at diagnosis of MCT, tumor aggressiveness as measured by the TNM classification, and basal calcitonin secretion with individual RET protooncogene mutations has not yet been undertaken. The current investigation provides evidence for the first time that there is a clear, probably genetically encoded, hierarchy of RET protooncogene mutations with respect to the onset of hereditary MTC. Obviously, germline mutations in the cysteine domain of exon 11 (codon 634) engender an extremely strong activation of the RET protooncogene, accounting for

TABLE 3. Age at diagnosis of medullary thyroid carcinoma grouped by RET genotype

Exon	Codon	Nucleotide exchange	Amino acid exchange	Patients [n/N (%)]	Families n/N (%)	Median age at diagnosis (25/75% quartiles)
10	611	TGC→TAC	Cys→Tyr	2/63 (3)	1/38 (3)	43 (42–44)
10	611	TGC→TTC	Cys→Phe	1/63 (2)	1/38 (3)	45 (–)
10	618	TGC→AGC	Cys→Ser	4/63 (6)	2/38 (5)	35 (13–62)
10	618	TGC→GGC	Cys→Gly	4/63 (6)	1/38 (3)	18 (13–33)
10	618	TGC→TTC	Cys→Phe	1/63 (2)	1/38 (3)	56 (–)
10	620	TGC→AGC	Cys→Ser	1/63 (2)	1/38 (3)	38 (–)
10	620	TGC→CGC	Cys→Arg	1/63 (2)	1/38 (3)	34 (–)
11	634	TGC→CGC	Cys→Arg	17/63 (27)	11/38 (29)	27 (8–47)
11	634	TGC→GGC	Cys→Gly	1/63 (2)	1/38 (3)	27 (–)
11	634	TGC→TAC	Cys→Tyr	8/63 (13)	4/38 (11)	29 (18–39)
11	634	TGC→TTC	Cys→Phe	3/63 (5)	2/38 (5)	34 (11–63)
11	634	TGC→TCC	Cys→Ser	5/63 (8)	3/38 (8)	23 (10–32)
13	768	GAG→GAC	Glu→Asp	4/63 (6)	2/38 (5)	60 (48–73)
13	790	TTG→TTC	Leu→Phe	4/63 (6)	3/38 (8)	57 (36–62)
13	790	TTG→TTT	Leu→Phe	5/63 (8)	3/38 (8)	33 (15–54)
14	804	GTG→ATG	Val→Met	2/63 (3)	1/38 (3)	62 (60–63)

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the very early onset of MTC and the frequent MEN IIA phenotype. Germline mutations in the cysteine domains of exon 10 (codons 609, 611, 618, and 620) seemingly entail a lesser activation of the RET protooncogene, resulting in later malignant transformation and in both the MEN IIA and FMTC phenotypes. In contrast, germline mutations in noncysteine domains of exons 13 and 14 (codons 768, 790, and 804) afford a weaker activation, resulting in a seemingly attenuated form with late-onset MTC and the FMTC phenotype only (2, 22). The clinical distinction between MEN IIA and FMTC may thus be arbitrary, merely reflecting the intensity of RET protooncogene activation and hence disease penetrance and expression (23). It is conceivable that the genetically encoded degree of RET protooncogene activation also accounts for the striking disparity between codons in the penetrance of pheochromocytoma and parathyroid hyperplasia. These accompanying tumors have been reported in approximately 50% and 10% of patients, respectively, associated with codon 634 mutations. Some FMTC patients with low activity mutations in exon 13 and 14 (*e.g.* in codons 768, 790, and 804) may simply not live long enough to see any of these tumors develop in their lifetime. The former concept of FMTC being restricted to exons 13 and 14 mutations (2) was not falsified until recently when Nilsson and co-workers (24) reported an association between pheochromocytoma and hereditary MTC in two of four family members with an exon 14 V804L germline mutation, suggesting an MEN IIa phenotype. The type of nucleotide and amino acid exchange apparently has no bearing on the pace of malignant transformation and hence on patient age at diagnosis of MTC. As pathological confirmation of MTC was a prerequisite for enrollment, patients with mutations in the high activity codon were recruited more frequently, raising their overall share of the study population. In keeping with the current investigation, Decker and associates (23) found codon 634 mutations in 51% of hereditary MTC, with the TGC→CGC missense change prevailing within this codon.

Interestingly, no correlations were encountered between RET protooncogene mutations and the pT, pN, or M category; the UICC stage; or basal serum calcitonin levels before surgery. These findings suggest that even a low transforming capacity, genetically encoded by a distinct RET protooncogene mutation, may be adequate for malignant progression and may ultimately lead to widespread metastatic MTC. Provided that tumor aggressiveness is genetically encoded, a correlation between the pT, pN, or M category; UICC tumor stage; and, to a lesser extent, the preoperative basal serum calcitonin level should have been found at least in the 36 index patients in whom the disease has naturally evolved without medical interference. In contrast, differences in oncological features that are genetically encoded are more difficult to detect in asymptomatic nonindex patients, as many of these RET gene carriers will today have thyroidectomy before an MTC will have emerged.

RET protooncogene mutations and transforming capacity on the cellular level

This idea is further supported by experimental data. When the transforming capacity of c-RET was examined after transfection into NIH-3T3 cells (25), Cys^{634} mutants had a 3- to 5-fold higher transforming capacity compared with exon 10 Cys mutants (codons 609, 611, 618, and 620). In this experimental model the transforming activity of each mutant protein was directly paralleled by the expression of RET on the cellular surface (25). Expression of RET codon 634 mutants was increased compared with that of 609, 611, 618, or 620 mutants. In addition, this experiment clearly indicated that different amino acid substitutions at any given Cys mutation of exon 10 (codons 609, 611, 618, and 620) yielded comparable transforming activity (25).

Clinical implications

The significant correlation between RET protooncogene mutation and patient age at diagnosis of MTC has important clinical implications. Based on youngest age at diagnosis, three groups can be devised by RET protooncogene mutation according to the speed of malignant transformation: a high risk group, encompassing codons 634 and 618, with youngest ages of 3 and 7 yr, respectively; an intermediate risk group, including codons 790, 620, and 611, with youngest ages of 12, 34, and 42 yr, respectively; and a low risk group, comprising codons 768 and 804, with youngest ages of 47 and 60 yr, respectively. Considering these data, it may be prudent to perform prophylactic thyroidectomy in the high risk group at 5 yr of age at the latest, in the intermediate risk group at age 10 yr, and in the low risk group at age 20 yr. Infants with high risk mutations below the age of 5 yr may have to undergo prophylactic surgery even earlier, *i.e.* upon demonstration of elevated basal or stimulated serum calcitonin levels. On the other hand, normal serum calcitonin does not reliably exclude MTC (1, 9). In line with this recommendation, Gill and co-workers (8) suggested yearly provocative screening beginning at age 1 yr and prophylactic thyroidectomy in MEN IIA patients as young as 5 yr after observing a 5-yr-old girl with MTC and nodal metastases and her 3-yr-old sister with MTC focus both harboring the C634R genotype (TGC→CGC; Cys→Arg). Some researches even proceed with prophylactic thyroidectomy as early as age 2 yr because MTC has been reported in MEN IIA at this young age (26). In the German and Austrian multicenter study on prophylactic thyroidectomy below the age of 20 yr, all three patients with lymph node metastases, aged 14, 15, and 19 yr, belonged to the high risk group, harboring codon 634 mutations. In our study no lymph node metastases were found below the age of 10 yr, which would argue against performing central lymph node dissection before that age on a prophylactic basis (9). In the present series the youngest patients with hereditary MTC and lymph node metastases was 9 yr old and not biochemically cured. The youngest patients with hereditary MTC and lymph node metastasis reported in the biomedical literature outside the MEN IIb setting were 5 and 6 yr of age (8, 27). For this reason, Skinner and co-workers (28) favor prophylactic thyroidectomy with central lymph node dissection at age 5 yr for MEN IIA patients.

Conclusion

A risk classification based on RET protooncogene genotype holds the promise of eliminating a nascent hereditary

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MTC by timely prophylactic thyroidectomy, *i.e.* before lymphatic or distant metastases have developed. Such a genetically driven approach would circumvent the increased morbidity attendant to central lymph node dissection (10) and ensure high rates of biochemical cure. This benefit obviously outweighs the lower quality of life scores in RET mutation carriers associated with knowledge about the genetic predisposition to cancer (29). Furthermore, the genetic approach is cost effective, as continuous biochemical screening is rendered superfluous in the majority of patients. An institutional approach, as presented in this article, offers the advantage of standardized genetic typing procedures, standardized operative strategies, and standardized biochemical testing using the same calcitonin assay and the same stimulatory agent. The limitations in patient numbers obviously are the most significant drawback of this approach. The median age at diagnosis of 62 yr in codon 804 mutations, which was based on just two patients in this institutional series, epitomizes this problem. Considering the fact that metastatic MTC has been observed at 30 and 32 yr of age in two families with V804M (GTG→ATG) germline mutations (30), this information might understate the malignant potential of this genotype in some patients and hence may be misleading, as it could lead to recommendations of a too late prophylactic thyroidectomy. With a view to further optimizing the timing of prophylactic thyroidectomy, a multicenter study is clearly needed to provide more exact data for the more common (codons 611, 618, 620, 634, 768, 790, and 804) and additional information on less frequent (codons 609, 630, 791, and 891) RET genotypes. To this end, standardization of genetic typing, operative strategies, and biochemical testing would be highly recommended.

References

1. **Wells SA, Chi DD, Toshima K, et al.** 1994 Predictive DNA testing and prophylactic thyroidectomy in patients at risk for multiple endocrine neoplasia type 2A. Ann Surg. 220:237–250.
2. **Eng C, Clayton D, Schuffenecker I, et al.** 1996 The relationship between specific RET proto-oncogene mutations and disease phenotype in multiple endocrine neoplasia type 2. JAMA. 276:1575–1579.
3. **Marsh DJ, Mulligan LM, Eng C.** 1997 RET proto-oncogene mutations in multiple endocrine neoplasia type 2 and medullary thyroid carcinoma. Horm Res. 47:168–178.
4. **Farndon JR, Leight GS, Dilley WG, et al.** 1986 Familial medullary thyroid carcinoma without associated endocrinopathies: a distinct entity. Br J Surg. 73:278–281.
5. **Donis-Keller H, Dou S, Chi D, et al.** 1993 Mutations in the RET proto-oncogene are associated with MEN 2A and FMTC. Hum Mol Genet. 2:851–856.
6. **Mulligan LM, Kwok JB, Healey CS, et al.** 1993 Germline mutations of the RET proto-oncogene in multiple endocrine neoplasia type 2A. Nature. 363:458–460.
7. **Decker RA, Geiger JD, Cox CE, Mackovjak M, Sarkar M, Peacock ML.** 1996 Prophylactic surgery for multiple endocrine neoplasia type 2a after genetic diagnosis: is parathyroid transplantation indicated?. World J Surg. 20:814–821.
8. **Gill JR, Reyes-Mugica M, Iyengar S, et al.** 1996 Early presentation of metastatic medullary carcinoma in multiple endocrine neoplasia type IIA: implications for therapy. J Pediatr. 129:459–464.
9. **Dralle H, Gimm O, Simon D, et al.** 1998 Prophylactic thyroidectomy in 75 children and adolescents with hereditary medullary thyroid carcinoma: German and Austrian experience. World J Surg. 22:744–751.
10. **Henry JF, Gramatica L, Denizot A, Kvachenyuk A, Puccini M, Defechereux T.** 1998 Morbidity of prophylactic lymph node dissection in the central neck area in patients with papillary thyroid carcinoma. Langenbeck Arch Surg. 383:167–169.
11. **Moers AMJ, Landsvater RM, Schaap C, et al.** 1996 Familial medullary thyroid carcinoma: not a distinct entity? Genotype-phenotype correlation in a large family. Am J Med. 101:635–641.
12. **Dralle H, Damm I, Scheumann GFW, et al.** 1994 Compartment oriented microdissection of regional lymph nodes in medullary thyroid carcinoma. Surg Today. 24:112–121.
13. **Hinze R, Holzhausen HJ, Gimm O, Dralle H, Rath FW.** 1998 Primary hereditary medullary thyroid carcinoma: C-cell morphology and correlation with preoperative calcitonin levels. Virchows Arch. 433:203–208.
14. **Sobin LH, Wittekind Ch, eds.** 1997 TNM classification of malignant tumours, 5th Ed. New York: Wiley-Liss.
15. **Berndt I, Reuter M, Saller B, et al.** 1998 A new hot spot for mutations in the *ret* protooncogene causing familial medullary thyroid carcinoma and multiple endocrine neoplasia type 2A. J Clin Endocrinol Metab. 83:770–774.
16. **Schneider R.** 1992 The human protooncogene *ret*: a communicative cadherin? Trends Biochem Sci. 17:468–469.
17. **Iwamoto T, Taniguchi M, Asai N, Ohkusu K, Nakashima I, Takahashi M.** 1993 cDNA cloning of mouse ret proto-oncogene and its sequence similarity to the cadherin superfamily. Oncogene. 8:1087–1091.
18. **Ullrich A, Schlessinger J.** 1990 Signal transduction by receptors with tyrosine kinase activity. Cell. 61:203–212.
19. **Santoro M, Carlomagno F, Romano A, et al.** 1995 Activation of RET as a dominant transforming gene by germline mutations of MENA and MEN 2B. Science. 267:381–383.
20. **Hanks SK, Quinn AM, Hunter T.** 1988 The protein kinase family: conserved features and deduced phylogeny of the catalytic domains. Science. 241:42–52.
21. **Eng C, Smith DP, Mulligan LM, Healey CS, Zvelebil MJ, Stonehouse TJ, et al.** 1995 A novel point mutation in the tyrosine kinase domain of the RET proto-oncogene in sporadic medullary thyroid carcinoma and a family with FMTC. Oncogene. 10:509–513.
22. **Bolino A, Schuffenecker I, Luo Y, et al.** 1995 RET mutations in exons 13 and 14 of FMTC patients. Oncogene. 10:2415–2419.
23. **Decker RA, Peacock ML.** 1997 Update on the profile of multiple endocrine neoplasia type 2a RET mutations. Practical issues and implications for genetic testing. Cancer. 80:557–568.
24. **Nilsson O, Tisell LE, Jansson S, Ahlman H, Gimm O, Eng C.** 1999 Adrenal and extra-adrenal pheochromocytomas in a family with germline RET V804L mutation. JAMA. 281:1587–1588.
25. **Ito S, Iwashita T, Asai N, et al.** 1997 Biological properties of RET with cysteine mutations correlate with multiple endocrine neoplasia type 2A, familial medullary thyroid carcinoma, and Hirschsprung's disease phenotype. Cancer Res. 57:2870–2872.
26. **Telander RL, Zimmerman D, Sizemore GW, van Heerden JA, Grant CS.** 1989 Medullary carcinoma in children. Results of early detection and surgery. Arch Surg. 124:841–843.
27. **Graham SM, Genel M, Touloukian RJ, Barwick KW, Gertner JM, Torony C.** 1987 Provocative testing for occult medullary carcinoma of the thyroid: findings in seven children with multiple endocrine neoplasia type IIa. J Pediatr Surg. 22:501–503.
28. **Skinner MA, DeBenedetti MK, Moley JF, Norton JA, Wells SA.** 1996 Medullary thyroid carcinoma in children with multiple endocrine neoplasia types 2A and 2B. J Pediatr Surg. 31:177–82.
29. **Freyer G, Dazord A, Schlumberger M, et al.** 1999 Psychosocial impact of genetic testing in familial medullary thyroid carcinoma: a multicentric pilot-evaluation. Ann Oncol. 10:87–95.
30. **Feldman GL, Edmonds MW, Ainsworth PJ, et al.** 2000 Variable expressivity of familial medullary thyroid carcinoma (FMTC) due to a RET V804M (GTG→ATG) mutation. Surgery. 128:93–98.

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