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CLINICAL STUDY

Molecular epidemiology of multiple endocrine neoplasia 2: implications for RET screening in the new millenium

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Abstract

Objective: Twenty years ago, the groundbreaking discovery that rearranged during transfection (*RET*) mutations underlie multiple endocrine neoplasia 2 (MEN2) and familial medullary thyroid cancer (FMTC) ushered in the era of personalized medicine. MEN2-associated signs, taking time to manifest, can be subtle. This study sought to clarify to what extent conventional estimates of 1:200 000–500 000 underestimate the incidence of *RET* mutations in the population.
Design: Included in this retrospective investigation were 333 RET carriers born between 1951 and 2000 and operated on at the largest German surgical referral center (286 carriers) or elsewhere (47 carriers).
Methods: To estimate the incidence of *RET* mutations, the number of RET carriers born in Germany in five decades (1951–1960, 1961–1970, 1971–1980, 1981–1990, and 1991–2000) was divided by the corresponding number of German live births.
Results: Owing to improved diagnosis and capture of FMTC and MEN2 patients, minimum incidence estimates increased over time: overall from 5.0 (1951–1960) to 9.9 (1991–2000) per million live births and year ($P=0.008$), and by American Thyroid Association/ATA class from 1.7 to 3.7 for ATA class C ($P=0.008$); from 1.8 to 2.7 for ATA class A ($P=0.017$); from 1.5 to 2.2 for ATA class B ($P=0.20$); and from 0 to 1.4 for ATA class D mutations per million live births and year ($P=0.008$). Based on 1991–2000 incidence estimates the prevalence in Germany is ~1:80 000 inhabitants.
Conclusions: The molecular minimum incidence estimate of ≈1:100 000 was two- to fivefold greater than conventional estimates of 1:200 000–500 000.

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Introduction

Twenty years ago, the groundbreaking discovery that activating rearranged during transfection (*RET*) mutations in codons 609, 611, 618, 620, 630 (American Thyroid Association/ATA class B), 634 (ATA class C), and 918 (ATA class D) underlie multiple endocrine neoplasia type 2 (MEN2) and familial medullary thyroid cancer (FMTC) ushered in the era of personalized medicine (1, 2, 3, 4). The *RET* gene encodes for the membrane-bound RET receptor tyrosine kinase, which is mainly expressed in neural crest-derived cell lineages such as parafollicular C cells, adrenal medullary cells, and parathyroid chief cells (3). Clinically, MEN2 is subdivided into MEN type 2A (MEN2A), comprising medullary thyroid cancer (MTC), pheochromocytoma and primary hyperparathyroidism, and MEN type 2B (MEN2B). MEN2B does not encompass parathyroid adenoma/hyperplasia but includes ocular, oral, facial, and gastrointestinal ganglioma/ganglioneuroma and skeletal malformations instead. In the following 5 years, additional *RET* mutations were identified in codons 768, 790, 791, 804, and 891 (ATA class A) that almost invariably cause FMTC (4).

These seminal detections, representing an incremental scientific advance at the etiological level, allowed a glimpse at the codon-specific, age-related progression from C-cell hyperplasia to node-negative and node-positive MTC (5, 6) and the development of pheochromocytoma (7, 8) and parathyroid adenoma/hyperplasia (8, 9) in carriers of activating *RET* mutations. Although the intensity of C-cell stimulation is genetically encoded and more predictable, the acquisition of somatic mutations by a single C-cell is difficult to anticipate as these 'second hits' follow stochastic principles subject to the law of chance. The stronger is the transforming activity of a *RET* mutation *in vitro*, the faster is the development of MTC (10). For extracellular *RET* mutations, the transforming activity is the weaker

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(codon 609/611 < 618/620 < 630/634) the farther these mutations are positioned away from the cell membrane (10, 11). Because the transition from C-cell hyperplasia to node-negative and ultimately node-positive MTC takes time, the above histopathological stages are separated by time intervals (5, 6). This time lag of malignant transformation and tumor cell spread represents a 'window of opportunity' for surgical intervention before the tumor extends beyond the confines of the thyroid gland, rendering it much harder, if not impossible, to clear. For carriers of *RET* mutations in codon 634, this time interval was estimated at 6.9 years based on the mean age difference between patients with node-negative (10.2 years) and patients with node-positive thyroid cancer (17.1 years) (5). In contrast to other hereditable conditions, effective surgical interventions are available for mitigation because the affected endocrine organs are expendable, enabling prophylactic thyroidectomy, or paired, permitting therapeutic adrenalectomy and/or parathyroidectomy.

For genetic association studies, stringent criteria are essential to ensure that patients who meet the clinical case definitions have the disease of interest. This requirement explains why stronger *RET* mutations were overrepresented in early genetic studies of MEN2: mutations in codon 918 (ATA class D), accounting for >95% of MEN2B, and mutations in codon 634 (ATA class C), prevailing in MEN2A (1). Associated with moderate transforming activities, *RET* mutations in codons 609, 611, 618, 620, and 630 (ATA class B) went underreported in these series, and weaker *RET* mutations in codons 768, 790, 791, 804, and 891 (ATA class A) were missed altogether.

For a disease taking years, if not decades, to manifest, correct ascertainment of all patients at the population level is challenging. Largely based on clinical case definitions, the annual incidence of *RET* germline mutations has been estimated at 1:500 000 (12) and more recently at 1:200 000 (13), without detailing the rationale behind these estimates. Founded on molecular analysis of the *RET* proto-oncogene, the actual incidence of *RET* mutations is set to be higher because *RET* gene carriers who have not yet developed the disease are no longer missed (14). To test this hypothesis, the present investigation was undertaken at the largest surgical referral center for RET carriers in Germany.

Materials and methods

RET population

For the purpose of this retrospective investigation, the study base included 464 carriers of missense germline mutations in codons 609, 611, 618, 620, 630, 634, 768, 790, 791, 804, 891, or 918 of the *RET* proto-oncogene. Among these 464 patients, 400 carriers underwent standard MEN2-related interventions at this institution between November 1994 and October 2012 according to national and international practice guidelines and 64 additional carriers at other facilities. These 64 relatives, having been captured in the process of taking the patients' histories, had information on the year of birth. Foreign-born RET carriers traveling to Germany for surgical care were not considered for estimation of incidence and prevalence rates of *RET* germline mutations. For retrospective analysis of existing data sets from routine patient care, no institutional review board approval is required under German law and applicable institutional regulations.

RET gene analysis

Before undergoing genetic testing before or after the operation, all patients or their legal guardians had given informed consent after genetic counseling. For identification of relevant *RET* mutations, genomic DNA was purified from peripheral blood leukocytes using standard techniques. Genomic DNA was amplified using PCR and oligonucleotide primers for exons 8, 10, 11, 13, 14, 15, and 16. Single-strand conformation polymorphism analysis and direct sequencing were performed according to national laboratory and genetic regulations for *RET* analysis (6).

Calculating minimum incidence and prevalence rates of RET germline mutations

The geographic area of interest was the reunified Germany, comprising the territories of the former Federal Republic of Germany and the former German Democratic Republic. To yield more stable estimates, all RET carriers were excluded who were born before 1951 (some of these carriers may have died undiagnosed before MTC and MEN2 were defined as entities in their own right during the 1960s) or after 2000 (not all pediatric RET carriers may have yet been referred for prophylactic thyroidectomy). All remaining 333 RET carriers (286 institutional patients and 47 next of kin seen elsewhere) were assigned to one of five consecutive birth cohorts: 1951–1960, 1961–1970, 1971–1980, 1981–1990, and 1991–2000. Annual live birth data for Germany were provided by the Information Service of the German Federal Statistics Office (15). To calculate minimum incidence rates, the number of RET carriers in each decade was divided by the number of live births in Germany registered for the respective decade. The linear-by-linear association χ^2 statistic was calculated to evaluate temporal trends of these incidence rates overall and for each mutational category (ATA class). Minimum and maximum prevalence rates of *RET* mutations were approximated multiplying incidence estimates by mean life expectancy (60, 70, and 80 years for ATA class A–C mutations and 20, 30, and 40 years for ATA class D mutations).

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Results

Minimum incidence of RET germline mutations

Table 1, drawing on 333 RET carriers born between 1951 and 2000, depicts minimum incidence estimates of *RET* mutations in codons 918 (ATA class D), 634 (ATA class C), 630, 620, 618, 611, and 609 (ATA class B), and 768, 790, 791, 804, and 891 (ATA class A) by decade. Owing to improved diagnostic tests and more complete capture of FMTC and MEN2 patients, incidence estimates gradually increased over time: by individual *RET* mutation; by ATA class from 1.7 to 3.7 for ATA class C ($P=0.008$); from 1.8 to 2.7 for ATA class A ($P=0.017$); from 1.5 to 2.2 for ATA class B ($P=0.20$); and from 0 to 1.4 for ATA class D mutations per million live births and year ($P=0.008$); and overall (from 5.0 to 9.9 per million live births and year; $P=0.008$). Over the past 50 years, the rank order of these mutational ATA classes (C>A>B>D) changed little.

ATA class B mutations in codons 620 and 618 were almost twice as common as mutations in codons 611 and 609 (0.6–0.8 vs 0.4 per million live births and year). In contrast, ATA class A mutations in codons 768, 791, 804, and 891 were equally frequent (0.3–0.5 per million live births and year), with the exception of mutations in codon 790, which occurred more than twice as often (1.1 per million live births and year). Because a large number of pediatric RET carriers in Germany are being seen at this institution, at least half of all German RET carriers born between 1991 and 2000 should have been captured.

Prevalence of RET mutations in Germany

Compared with the general population, RET carriers diagnosed at an older age are presumed to have decreased life expectancies due to metastatic MTC or pheochromocytoma-associated hypertensive crisis (4). In the absence of a comprehensive national MEN2 registry recording molecular genetic in addition to clinical information, minimum and maximum prevalence rates of *RET* mutations were approximated multiplying incidence estimates by mean life expectancy (60, 70, and 80 years for ATA class A–C mutations and 20, 30, and 40 years for ATA class D mutations). As depicted in Table 2, some 1000 RET carriers (no fewer than 544–744 and no more than 1088–1488) should be living in Germany, a country with a population of 80 million inhabitants, translating into a prevalence of ≈1:80 000. Only a fraction of these 1000 RET carriers, meeting the RECIST criterion of a measurable target lesion ≥1 cm in greatest dimension, may be eligible for clinical trials. Our estimates yielded a minimum of 222–296 carriers of *RET* missense mutations in codon 634, 166 of whom are documented at our tertiary referral center. These extrapolations compared favorably with early estimates from the 1990s that up to 1500 RET carriers from up to 300 RET families may be living in Germany (16).

Geographic distribution of RET *mutations in continental Europe*

In Italy, France, and Germany, genetic counseling and molecular *RET* gene analysis are provided free of charge for the next of kin of FMTC and MEN2 families and

Table 1 Minimum incidence of *RET* missense germline mutations in Germany 1951–2000. Owing to rounding, not all numbers add up.

		10-Year birth cohorts (333 carriers)					
		1951–1960 (11 513 774 live births)	1961–1970 (12 663 974 live births)	1971–1980 (8 414 287 live births)	1981–1990 (8 572 731 live births)	1991–2000 (7 903 367 live births)	
ATA class	**Mutated RET codon**	Carriers, *n* (per 1 million and year)	Carriers, *n* (per 1 million and year)	Carriers, *n* (per 1 million and year)	Carriers, *n* (per 1 million and year)	Carriers, *n* (per 1 million and year)	***P* for trend**
D	*M918T*	*0*	*4 (0.3)*	*5 (0.6)*	*10 (1.2)*	*11 (1.4)*	*0.008*
C	*C634R/G/F/S/W/Y*	*19 (1.7)*	*30 (2.4)*	*24 (2.9)*	*17 (2.0)*	*29 (3.7)*	*0.008*
B	C630R/F/S/Y	0	1 (0.1)	1 (0.1)	0	0	
	C620R/G/F/S/W/Y	2 (0.2)	6 (0.5)	5 (0.6)	5 (0.6)	5 (0.6)	
	C618R/G/F/S/Y	8 (0.7)	5 (0.4)	4 (0.5)	8 (0.9)	6 (0.8)	
	C611R/G/F/S/W/Y	7 (0.6)	3 (0.2)	7 (0.8)	2 (0.2)	3 (0.4)	
	C609R/F/S/Y	0	2 (0.2)	0	0	3 (0.4)	
	Any	*17 (1.5)*	*17 (1.3)*	*17 (2.0)*	*15 (1.7)*	*17 (2.2)*	*0.20*
A	E768D	1 (0.1)	2 (0.2)	0	0	2 (0.3)	
	L790F	6 (0.5)	9 (0.7)	2 (0.2)	8 (0.9)	9 (1.1)	
	Y791F	5 (0.4)	2 (0.2)	0	6 (0.7)	3 (0.4)	
	V804L/M	8 (0.7)	10 (0.8)	11 (1.3)	6 (0.7)	3 (0.4)	
	S891A	1 (0.1)	0	1 (0.1)	2 (0.2)	4 (0.5)	
	Any	*21 (1.8)*	*23 (1.8)*	*14 (1.7)*	*22 (2.6)*	*21 (2.7)*	*0.017*
Total	Any	57 (5.0)	74 (5.8)	60 (7.1)	64 (7.5)	78 (9.9)	0.008

ATA, American Thyroid Association; RET, rearranged during transfection.

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Table 2 Estimated prevalence of *RET* gene carriers in Germany.

ATA class	Mutated RET codon	Minimum *RET* gene incidence per 1 million and year (Table 1; 1991–2000)	Prevalence of German *RET* gene carriers, *n*[a] Minimum (−Maximum)[b] Scenario 1 (conservative): mean life expectancy: 60 years (ATA class A–C) 20 years (ATA class D)	Scenario 2 (reasonable): mean life expectancy: 70 years (ATA class A–C) 30 years (ATA class D)	Scenario 3 (optimistic): mean life expectancy: 80 years (ATA class A–C) 40 years (ATA class D)
D	*M918T*	*1.4*	*28 (−56)*	*42 (−84)*	*56 (−112)*
C	*C634R/G/F/S/W/Y*	*3.7*	*222 (−444)*	*259 (−518)*	*296 (−592)*
B	C630R/F/S/Y	(0.1)[c]	(6 (−12))[c]	(7 (−14))[c]	(8 (−16))[c]
	C620R/G/F/S/W/Y	0.6	36 (−72)	42 (−84)	48 (−96)
	C618R/G/F/S/Y	0.8	48 (−96)	56 (−112)	64 (−128)
	C611R/G/F/S/W/Y	0.4	24 (−48)	28 (−56)	32 (−64)
	C609R/F/S/Y	0.4	24 (−48)	28 (−56)	32 (−64)
	Any	*2.2*	*132 (−264)*	*154 (−308)*	*176 (−352)*
A	E768D	0.3	18 (−36)	21 (−48)	24 (−48)
	L790F	1.1	66 (−132)	77 (−154)	88 (−176)
	Y791F	0.4	24 (−48)	28 (−56)	32 (−64)
	V804L/M	0.4	24 (−48)	28 (−56)	32 (−64)
	S891A	0.5	30 (−60)	35 (−70)	40 (−80)
	Any	*2.7*	*162 (−324)*	*189 (−378)*	*216 (−432)*
Total	Any	9.9	544 (−1088)	644 (−1288)	744 (−1488)

ATA, American Thyroid Association; RET, rearranged during transfection.
[a]Prevalence equals incidence multiplied by average life expectancy, based on an estimated 1 million live births per year.
[b]Maximum prevalence is estimated at no more than twice the minimum prevalence observed in the most recent decade 1991–2000 (Table 1).
[c]1971–1980 estimate (Table 1), not used for prevalence calculations of ATA class B *RET* gene carriers.

patients diagnosed with MTC. Under these singular conditions, activating missense *RET* mutations are distributed fairly even across these three countries (Table 3) (17, 18, 19, 20). Possible exceptions include mutations: in codon 790, being three times more common in German than Italian and French RET families (13 vs 4%); in codon 804, being two- to threefold more frequent in Italian and French than German RET families (15–26 vs 9%); and in codon 918 (16 vs 3–9%), perhaps reflecting this institution's surgical specialization.

Based on 500 Continental European RET families, the proportion of RET frequencies was as follows: 34.2% for codon 634, 17.2% for codon 804, 10.4% for codon 918, 7.6% for codon 790, 7.2% for codon 891, 7.0% for codon 620, 6.4% for codon 618, 3.2% for codon 791, 2.6% for codon 768, 1.6% each for codons 609 and 611, and 1.0% for codon 630 mutations. Among these

Table 3 Distribution of *RET* missense germline mutations in Continental Europe (500 RET families)[a]. Owing to rounding, not all numbers add up.

ATA class	*RET* mutation	Germany, Halle 1994–2012[b] *n* (%)	Italy, multicenter (18) *n* (%)	France, multicenter (19, 20) *n* (%)	Total *n* (%)
D	M918T	32 (16)	17 (9)	3 (3)	52 (10.4)
D	A883F	0	0	0	0
C	C634R/G/F/S/W/Y	73 (36)	52 (26)	46 (47)	171 (34.2)
B	C630R/F/S/Y	1 (0.5)	4 (2)	0	5 (1.0)
B	C620R/G/F/S/W/Y	14 (7)	9 (5)	12 (12)	35 (7.0)
B	C618R/G/F/S/Y	11 (5)	15 (8)	6 (6)	32 (6.4)
B	C611R/G/F/S/W/Y	6 (3)	1 (1)	1 (1)	8 (1.6)
B	C609R/F/S/Y	1 (0.5)	6 (3)	1 (1)	8 (1.6)
A	G533C	0	0	0	0
A	E768D	2 (1)	9 (5)	2 (2)	13 (2.6)
A	L790F	26 (13)	8 (4)	4 (4)	38 (7.6)
A	Y791F	14 (7)	2 (1)	0	16 (3.2)
A	V804L/M	19 (9)	52 (26)	15 (15)	86 (17.2)
A	S891A	6 (3)	23 (12)	7 (7)	36 (7.2)
Total	Any	205 (100)	198 (100)	97 (100)	500 (100)

ATA, American Thyroid Association; RET, rearranged during transfection.
[a]Considering series with a minimum of 30 European RET families only that specified familial RET prevalence.
[b]Updated from reference (17) (141 RET families).

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500 RET families there were no carriers of heterozygous *RET* mutations in codons 533 or 883 (i.e. A883F; disregarding the exceptional A883T mutation causing FMTC merely in homozygous, not in heterozygous, condition (21)). Germline mutations in these two codons have been reported from Brazil (22) and Greece (23) and the USA (24). A total of 177 and 284 patients with clinically sporadic MTC seen at this institution underwent molecular scanning specifically for codons 533 and 833 respectively, which was always negative.

Geographic distribution of RET mutations outside Europe

At the global level, national health care systems differ greatly regarding the availability of, easy access to, and the comprehensiveness of *RET* gene analysis which national estimates of incidence and prevalence depend on. These disparities can have a serious impact on a health care system's ability to unearth *RET* mutations in a given country. For many countries, including the USA and Australia, estimates regarding the distribution of familial *RET* mutations have not yet been put forward.

Outside the Western hemisphere, *RET* gene analysis used to be employed predominantly for molecular confirmation of clinically manifest MEN2A and 2B, for instance in Argentina (25), Iran (26), India (27), Korea (28), and China (29, 30). In this setting, stronger *RET* mutations in codons 634 (ATA class C) and 918 (ATA class D) rule the scene (64–100%, as opposed to 45% in Continental Europe) and moderate (ATA class B) or weak (ATA class A) mutations are exceptional (Table 4). This situation may be changing rapidly in some regions with the rollout of extensive screening programs (30).

In Central European countries where statutory health insurance plans cover genetic counseling services and *RET* gene analysis (31), ATA class A (189 of 500 RET families, or 37.8%) and ATA class B mutations (88 of 500 RET families, or 17.6%) comprise more than half of all *RET* mutations (Table 3). Intermediate positions occupy countries like the USA where easy access is given to *RET* gene analysis but not every patient has adequate health insurance.

Discussion

Molecular epidemiology examines the contribution of genetic risk factors detected on the molecular level to the etiology, distribution, and prevention of disease. This branch of medical science is heavily reliant on comprehensive population-based data that capture molecular information together with histopathological and clinical outcome data.

Limitations of the study

By implication, the incidence and prevalence of germline mutations in a given population cannot be exactly known without the systematic collection and storage of molecular genetic information in a population-based nationwide registry. Even under quasi-ideal conditions, the possibility remains that subjects carrying mutations with low transforming activity may not be captured because they do not yield pertinent family histories and have not yet developed MTC. The absence of population-based registries may reflect the lack of a societal consensus regarding the use of genetic data and fear of genetic discrimination. Based on the recent 1991–2000 birth cohort to minimize the effects of sampling bias, our incidence estimate of at least 1:100 000 for *RET* germline mutations is reliable because molecular genetic screening, barring sample

Table 4 Prevalence of *RET* missense germline mutations around the world (89 RET families)[a].

ATA class	*RET* mutation	Argentina (25) *n* (%)	Iran (26) *n* (%)	India (27) *n* (%)	Korea (28) *n* (%)	China (29) *n* (%)	China (30) *n* (%)	Total *n* (%)
D	M918T	5 (24)	1 (9)	2 (13)	0 (0)	5 (25)	0	13 (15)
D	A883F	0	0	0	0	0	0	0
C	C634R/G/F/S/W/Y	16 (76)	6 (55)	9 (60)	10 (77)	15 (75)	8 (80)	64 (72)
B	C630R/F/S/Y	0	1 (9)	0	0	0	0	1 (1)
B	C620R/G/F/S/W/Y	0	0	0	0	0	0	0
B	C618R/G/F/S/Y	0	1 (9)	1 (7)	2 (23)	0	1 (10)	5 (6)
B	C611R/G/F/S/W/Y	0	1 (9)	0	0	0	0	1 (1)
B	C609R/F/S/Y	0	0	2 (13)	0	0	0	2 (2)
A	G533C	0	0	0	0	0	0	0
A	E768D	0	0	0	0	0	0	0
A	L790F	0	0	0	0	0	1 (10)	1 (1)
A	Y791F	0	0	0	0	0	0	0
A	V804L/M	0	1 (9)	1 (7)	0	0	0	2 (2)
A	S891A	0	0	0	0	0	0	0
Total	Any	21 (100)	11 (100)	15 (100)	12 (100)	20 (100)	10 (100)	89 (100)

ATA, American Thyroid Association; RET, rearranged during transfection.
[a]Considering series with a minimum of ten non-European RET families only that specified familial RET prevalence.

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mix-up, is accurate (3). In our experience, relatives of established RET carriers rarely, if ever, refuse *RET* gene analysis. More patients presenting with seemingly sporadic MTC may subsequently emerge as index patients carrying the least penetrant ATA class A mutations. In that event, our minimum incidence estimate based on the 1991–2000 birth cohort would continue to rise. Less dependable is our prevalence estimate of 1:80 000 because life expectancies of patients carrying ATA class A, B, C, and D mutations, presumably improving as a result of earlier diagnosis, may not remain constant over time. Our prevalence estimate of 1:80 000 for hereditary C-cell disease was 37.5% of the overall MTC prevalence of 1:30 000 in the literature. In keeping, the percentage of hereditary MTC was 37% (101 patients) among the 270 patients operated on at this institution for MTC newly diagnosed between 1991 and 2000.

Molecular vs clinical incidence and prevalence of RET mutations

For an age-dependent hereditable disease, molecular epidemiology can yield more accurate incidence estimates than clinical epidemiology that, by definition, hinges on the clinical manifestation of the condition. Some RET carriers may even die of unrelated causes before developing MEN2-associated symptoms. Remarkably, our minimum estimate of 9.9 per million live births and year (Table 1), or ≈1:100 000, was fivefold greater than the traditional estimate of 1:500 000 (12) and twofold greater than the more recent estimate of 1:200 000 (13). These earlier estimates may derive from clinically apparent MEN2A and MEN2B patients carrying *RET* mutations in codons 634 (ATA class C) and 918 (ATA class D): 1.7 per million live births and year (our 1951–1960 data in Table 1), ∼1:500 000; and 5.1 per million live births and year (our 1991–2000 data in Table 1) or ≈1:200 000, the highest estimate available from the literature (13).

Implications for RET screening programs in the new millenium

Because screening is defined as the systematic examination of a group of asymptomatic individuals to identify those with a high probability of having a given disease, screening for *RET* mutations must include all relevant exons of the *RET* gene, at least exons 10, 11, 13, 14, 15, and 16. Before more data are forthcoming from other countries, exon 8 may need to be scanned in addition – not just in Greek patients presenting with apparently sporadic MTC, 7.8% (ten of 129) of whom may carry mutations in codon 533 (23).

Attempting to strike a balance between the cost and effectiveness of screening programs, ATA recommendation 11 states that 'analysis of the MEN 2-specific exons of RET is the recommended method of initial testing in either a single or multi-tiered approach' (2). This recommendation does not provide guidance on what criteria in a multi-tiered approach trigger a subsequent request for additional screening of exons 13, 14, and 15 in a patient who appears to have sporadic MTC, the usual first presentation of ATA class A mutations. These mutations are not as innocuous as frequently portrayed. As a matter of fact, distant metastases occurred in 13–19% of index patients (two of 16 and three of 16 RET carriers respectively) who carried *RET* mutations in codon 804 or 790 (32). For nonindex patients, the corresponding rates were 0% (0 of 34 carriers) and 3% (one of 31 carriers), confirming the usefulness of systematic RET screening.

Barriers to the utilization of RET screening

In our increasingly mobile society, different service providers may be caring for one individual at various stages in the disease process. This geographic dispersion, characterized by the disintegration of familial relationships with loss of medical information, strengthens the case of screening all patients with seemingly sporadic MTC for mutations in exons 10, 11, 13, 14, 15, and 16 as a minimum (4). Geographic dispersion can frustrate screening efforts directed at an index carrier's next of kin: of 86 members of a large French family carrying a *RET* mutation in codon 790, no more than 22 relatives (26%) agreed to be screened for the mutation running in the family (33). Moreover, not every *RET* gene carrier is prepared to share personal genetic information with his or her next of kin. As exemplified by two German RET families, this unwillingness can delay for many years recognition of the hereditable trait in other branches of a RET family (4). The reasons why certain gene carriers are not prepared to share genetic information with their relatives who run a substantial risk of developing MTC during their lifetimes remain to be clarified. Hypothetical reasons include repression, feelings of guilt and resentment, emotional distress, and poor familial interaction (4). Other obstacles are covert social and ethical barriers to genetic screening for MTC, which include literacy, education, income, culture/religion, social/family relationships, and inadequate decision-making capacity (14).

Future perspectives

The distribution of activating *RET* mutations, seemingly differing around the globe (Tables 3 and 4), is thought to primarily reflect the performance of the respective health care systems at the population level. This view is supported by a number of institutional reports of RET families harboring ATA class A mutations, e.g. in codon 533 from Brazil (22), in codons 768 and 804 from Japan (34), and in codons 804 and 891 from the USA (35). Many, if not all, of these differences in RET distribution may disappear once all relevant RET exons

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are being screened routinely in all patients presenting with MTC or MEN2. Regional differences in RET distribution that may exist are likely to diminish as a result of accelerating migration of people across geographic boundaries that used to be insurmountable.

Although a formal health technology assessment has not been conducted, targeted RET screening programs are probably more cost-effective than the recent suggestion that all newborns be indiscriminately evaluated for *RET* mutations in neonatal screening programs (14), which have a minimum overall incidence of 1 per 100 000 live births and year.

Declaration of interest

The authors declare that they have no conflicts of interests. K Frank-Raue, F Raue, and W Höppner operate privately owned molecular laboratories offering *RET* gene analyses, which are reimbursed by statutory and private health insurance programs in Germany.

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