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Risk Profiles and Penetrance Estimations in Multiple Endocrine Neoplasia Type 2A Caused by Germline *RET* Mutations Located in Exon 10

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ABSTRACT: Multiple endocrine neoplasia type 2 is characterized by germline mutations in *RET*. For exon 10, comprehensive molecular and corresponding phenotypic data are scarce. The International *RET* Exon 10 Consortium, comprising 27 centers from 15 countries, analyzed patients with *RET* exon 10 mutations for clinical-risk profiles. Presentation, age-dependent penetrance, and stage at presentation of medullary thyroid carcinoma (MTC), pheochromocytoma, and hyperparathyroidism were studied. A total of 340 subjects from

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103 families, age 4–86, were registered. There were 21 distinct single nucleotide germline mutations located in codons 609 (45 subjects), 611 (50), 618 (94), and 620 (151). MTC was present in 263 registrants, pheochromocytoma in 54, and hyperparathyroidism in 8 subjects. Of the patients with MTC, 53% were detected when asymptomatic, and among those with pheochromocytoma, 54%. Penetrance for MTC was 4% by age 10, 25% by 25, and 80% by 50. Codon-associated penetrance by age 50 ranged from 60% (codon 611) to 86% (620). More advanced stage and increasing risk of metastases correlated with mutation in codon position (609→620) near the juxtamembrane domain. Our data provide rigorous bases for timing of premorbid diagnosis and personalized treatment/prophylactic procedure decisions depending on specific *RET* exon 10 codons affected.
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KEY WORDS: MEN2; MEN2A; MEN2B; *RET*; medullary thyroid carcinoma; pheochromocytoma; genotype–phenotype

Introduction

Multiple endocrine neoplasia type 2 (MEN2) is an autosomal dominant multiglandular tumor syndrome, affecting derivatives from neural crest. MEN2 is caused by germline mutations of the *RET* proto-oncogene (MIM♯ 164761), encoding a transmembrane receptor tyrosine kinase expressed by the C-cells of the thyroid, adrenal medullary cells, and enteric autonomic ganglia [Elisei et al., 2007; Eng et al., 1996; Zbuk and Eng, 2007]. When *RET* mutations were found in the large majority of MEN2 patients and a *RET* genotype–clinical phenotype found [Eng et al., 1996], this was the paradigm for the practice of clinical cancer genetics [Kloos et al., 2009; Zbuk and Eng, 2007]. Accurate molecular diagnosis, premorbid predictive testing, and gene-informed medical management became possible.

Based on combinations of clinical manifestations, MEN2 is historically classified into three clinical subtypes: MEN2A (MIM♯ 171400), MEN2B (MIM♯ 162300), and familial medullary thyroid carcinoma (FMTC; MIM♯ 155240). The components of MEN2A are medullary thyroid carcinoma (MTC), pheochromocytoma, and parathyroid adenoma and/or hyperplasia (HPT). In MEN2B, constitutional abnormalities such as marfanoid habitus and neuromas of the tongue and the intestine are present, whereas clinical parathyroid disease is absent. FMTC refers to the familial occurrence of MTC without other lesions [Kloos et al., 2009]. MEN2B is considered more aggressive than MEN2A or FMTC, because the age-of-onset of the neoplasias is a mean 10 years earlier than that of MEN2A, and metastases from MTC occur as early as 3 years of age [Kloos et al., 2009].

The spectrum of germline *RET* mutations in MEN2 are encompassed by 64 distinct mutations that lie in 32 codons belonging to 8 exons [Kloos et al., 2009]. It is clear that the MEN2B-defining mutations, p.M918T and p.A883F, portend an aggressive course, with management appropriately aggressive and pre-emptive [Kloos et al., 2009]. It is also obvious and relatively consistent that the exon 11 C634R, the most common mutation seen in MEN2A, predicts for the development of MTC, pheochromocytoma, and HPT, at relatively young ages, and so management is tailored accordingly [Eng et al., 1996; Frank-Raue et al., 2006; Kloos et al., 2009; Neumann et al., 2002]. However, apart from vague generalizations such as non-634 mutations that are not as aggressive, and perhaps have later ages of onset and decreased penetrance, the breadth of clinical presentations and course, and the less-than-firm data preclude clinicians from altering medical management based on precise single-codon/specific missense mutation genotype. For instance, studies have shown that mutations at codon 634, the most frequently mutated in MEN2, result in a receptor tyrosine kinase that is constitutively active by the tendency of mutant *RET* monomers to undergo dimerization, a situation that simulates ligand binding. Whereas cysteine residues in wild-type *RET* form intramolecular disulfide bonds, mutation of a cysteine residue leaves an unpaired residue. Unpaired cysteines from two mutant *RET* monomers can dimerize by formation of a disulfide bond. Taking into account the clinical presentation, we know that the p.C634R mutation is highly penetrant and associated with the more severe forms of MEN2A [Eng, 1996; Kloos et al., 2009]. Similarly, while investigating pheochromocytoma presentations in our Registry, we noted that MTC does not always occur before other component neoplasias in those with p.C634W mutations [Neumann et al., 2007]. Therefore, we believe that single nucleotide genotype-specific clinical risk profiles comprising risk of developing each component neoplasia, the ages-at-onset, penetrance, and clinical course might be useful for tailoring more precise clinical care.

In contrast to well-defined risk profiles for carriers of the p.C634R, p.C634Y, p.C634W, p.A883F, and p.M918T mutations, mutations located in the codons of *RET* exon 10 are relatively rare [Mian et al., 2009; Moers et al., 1996; Siggelkow et al., 2001], and their anecdotal penetrance and ages of onset have resulted in appropriately cautious and tentative nonconsensual clinical guidelines [Brandi et al., 2001; Kloos et al., 2009]. Here, concerted effort from an international consortium have resulted in *RET* exon 10 genotyping, documented phenotypes at presentation, and longitudinal clinical data with the purpose of generating codon- and nucleotide-specific genotype-adjusted neoplasia risk profiles for exon 10 mutations.

Patients and Methods

Patients

The inclusion criterion for registrants was a proven carrier status of a germline mutation in exon 10 of the *RET* gene. In addition, relatives of index registrants were also registered if an MTC or a pheochromocytoma was diagnosed histologically. The ascertainment of carriers of such mutations was performed in three steps. First, *RET* exon 10 mutation carriers were identified from existing registrants in the European–American Pheochromocytoma Registry (EAPR), which has been previously described [Erlic et al., 2009; Neumann et al., 2002, 2004, 2007; Schiavi et al., 2005]. Second, all clinicians and researchers who had contributed to this registry or to our previous risk estimation study of *RET* p.C634W carriers were contacted [Milos et al., 2008]. Finally, all known centers with clinical activities in MEN2 or one of the component neoplasias (e.g., MTC or pheochromocytoma) were asked to contribute any relevant data to this study.

Clinical Data

Recording of demographic and clinical information was performed using a uniform data-format for all registrants. Demographic data included year of birth, gender, ethnic background, and country of residence. Clinical data were recorded for thyroid, adrenal and parathyroid glands, and additional features of MEN2, as follows.

For all three components, that is, MTC, pheochromocytoma and HPT, the age(s) at diagnosis were registered using the age at histological diagnosis. Criteria for absence of MTC included normal basal serum calcitonin or histological absence in surgical thyroid specimens. Absence of pheochromocytoma was documented either by normal CT or MRI of the adrenal glands or by normal 24-hr catecholamine excretion. Normal serum calcium and parathyroid hormone levels denoted an absence of hyperparathyroidism.

We differentiated symptomatic patients and subjects who were identified by screening procedures (screening assessment). Screening procedures included genetic screening of relatives after a *RET* mutation was identified and verified in an index case. This was followed by clinical screening if the mutation was also found in that relative. In addition, relatives may have already had clinical screening following the identification of MEN2 or one of its principal components in an index case.

For histopathology of the thyroid, we recorded presence or absence of C-cell hyperplasia and of MTC. For lymph node status, we recorded presence or absence of metastases at thyroid surgery. Distant metastases were recorded at thyroid surgery or any time during follow-up.

For MTC tumor stages, we used the TNM classification of 2002 and the AJCC classification [DeLellis et al., 2004; Greene et al., 2002; Sobin, 2002]. T1N0M0 for MTC <2 cm in diameter, T2N0M0 for MTC of 2–4 cm in diameter, T3 >4 cm either limited to the thyroid or with minimal invasion to the sternocleidomastoid muscle or perithyroidal soft tissue, T4a any size with invasion to subcutaneous tissue, larynx, trachea, esophagus, or recurrent laryngeal nerve, T4b invasion of prevertebral fascia, carotid artery, or mediastinal vessels, N1 for lymph node metastases and M1 for distant metastases. In cases of persistent or clearly elevated calcitonin levels after thyroidectomy including regional lymph node dissection, we classified six patients with distant metastases, although radiological evidence was not present.

This study has been approved by the respective institutional review boards for human subjects' protection in accordance with the ethical standards of each country and center.

Statistical Analysis

Categorical variables were summarized as frequency counts and percentages. Continuous variables were summarized as the median and range. MTC stage, cure/no-cure, and presence/absence of metastasis were compared among codons. Comparisons were made with the chi-square test, which determines if variables differ between at least two of the four codons, and with the Cochran-Mantel-Haenszel mean score test (TNM stage) or Cochran-Armitage trend test (cure, metastasis). These trend tests account for the ordering of codons by distance from the transmembrane domain and assess trends in stage, cure, or metastasis. Risk factors for MTC were assessed via chi-square tests and logistic regression analysis. Three candidate MTC risk factors were assessed: gender, age, and codon. All three variables were included in a multivariate logistic regression model whether or not they were statistically significant. Age-dependent penetrance estimates of MTC, pheochromocytoma, and HPT were calculated using the Kaplan-Meier method. Penetrance estimates were compared among codons using the log-rank test and with Cox proportional hazards analysis. The log-rank analysis assesses penetrance differences among codons, whereas the Cox analysis assesses trends across codons. Some patients had incomplete data regarding the different manifestations and were excluded from penetrance estimates. Thus, the penetrance data for MTC are based on 340 subjects, pheochromocytoma 319 subjects, and HPT 299 subjects. All analyses were done using SAS® software (SAS® Institute, Inc., Cary, NC). All statistical tests were two sided, and $P \leq 0.05$ was used to indicate statistical significance.

Results

Overall, 340 individuals (199 females, 141 males) had germline mutations in exon 10 of the *RET* gene and their clinical phenotypes and penetrance were analyzed for purposes of this study. The mutations affected only cysteine codons 609, 611, 618, and 620 (Table 1). The 340 subjects (255 European, 85 South American; comprising 280 of Caucasian, 20 of Hispanic, 3 of Italian-German-Hispanic, 37 of Indian-Caucasian, and 1 of Gypsy origin), belonged to 103 different families and hailed from 14 different countries (Table 1). Each of the centers contributed from 1 to 43 cases. The center specific distributions of cases amongst the codons is shown in Table 2.

Codon 609 mutations were found in 45 subjects from 13 families. Codon 611 mutations were noted in 50 subjects from 12 families. Codon 618 mutations were found in 94 subjects from 37 families. Codon 620 mutations were identified in 151 subjects from 41 families (Table 1).

Phenotypic Spectrum of *RET* Exon 10 Mutations

MTC was the most common neoplasia, with 263 affected (77%). In the remaining 23%, diagnosis of MTC was excluded by objective means. The median age at diagnosis of MTC was 35 (range: 4–86). Of note, symptomatic ascertainment of MTC occurred in 47% of the 263 patients whereas in 53%, their MTC was detected by screening.

Pheochromocytoma was documented in 54 of 319 subjects (17%). The median age at diagnosis of pheochromocytoma was 42 years (17–80 years). Pheochromocytoma was observed in individuals with mutations of all four codons. However, pheochromocytoma was not observed in carriers of 7 of the 22 distinct mutations. The frequencies of pheochromocytoma varied, occurring in 26% of those patients with codon 609 mutations, 10% codon 611, 23% codon 618, and 13% codon 620 (Table 1). All pheochromocytomas were adrenal in location. Bilateral pheochromocytoma was present in 15 of 54 (28%) affected subjects. None had malignant pheochromocytoma. Symptomatic pheochromocytoma occurred in 29 (54%) of the 54 patients; 24 of these 29 also had MTC. Among these 24, pheochromocytoma was diagnosed at least 1 year prior to MTC in 6 patients (25%), within 1 year of MTC in 8 (33%), and at least 1 year after MTC in the remaining 10 (42%).

HPT was present in 8 of 299 subjects (2.7%). Median age at diagnosis of HPT was 46 years (range: 28–82). Of the eight with HPT, seven were ascertained by screening at an asymptomatic stage. Although sample size is small, HPT was found in 4 out of 22 distinct mutations residing in all four exon 10 codons.

Regarding codon-specific neoplasias seen in our MEN2 series, MTC and pheochromocytoma were present in carriers of germline mutations of each of the four codons. Notably, codon 609 mutation carriers as a group have similar frequencies of MTC and pheochromocytoma as the groups carrying mutations in codons 611, 618, and 620 ($P = 0.2$, Fisher two-tailed exact test). However, if we analyzed the frequencies of MTC and pheochromocytoma for each distinct mutation, FMTC was found in patients carrying six specific missense mutations distributed across all four codons

Table 1. Demographic and Molecular Data of the Study Population

Codon	Nucleotide	Protein	Families *n*	Carriers *n*	Gender f/m	Age at MTC diagnosis: median (range)	MTC *n* (%)	Pheo *n* (%)	HPT *n* (%)	HSCR *n* (%)	Phenotype	Age at N1M0 *n* (range)	Age at M1 *n* (range)
609	c.1825T>C	p.C609R	2	7	7/0	42 (29–43)	4/7 (57)	2/7 (29)	0	0	MEN2A	1 (40)	0
	c.1825T>G	p.C609G	3	6	1/5	48 (4–54)	5/6 (83)	4/5 (80)	0	0	MEN2A	2 (33–48)	0
	c.1826G>A	p.C609Y	4	8	7/1	33 (21–49)	7/8 (88)	0	0	2/8 (25)	FMTC, HSRC	2 (21–48)	0
	c.1826G>C	p.C609S	2	18	6/12	37 (15–86)	14/18 (78)	4/17 (24)	2/17 (12)	0	MEN2A	1 (38)	1 (62)
	c.1826G>T	p.C609F	2	6	5/1	45 (36–63)	4/6 (67)	1/6 (17)	0	0	MEN2A	1 (54)	0
	All		**13**	**45**	**26/19**	**37 (4–86)**	**34/45 (76)**	**11/43 (26)**	**2/44 (5)**	**2/37 (5)**	**MEN2A, HSRC**	**7 (21–54)**	**1 (62)**
611	c.1832G>A	p.C611Y	7	15	11/4	35 (15–65)	9/15 (60)	4/15 (27)	1/15 (7)	0	MEN2A	4 (29–64)	1 (65)
	c.1833C>G	p.C611W	2	5	4/1	37 (14–66)	5/5 (100)	1/5 (20)	0	0	MEN2A	1 (49)	1 (37)
	c.1832G>T	p.C611F	2	26	12/14	44 (25–69)	19/26 (73)	0	0	0	FMTC	1 (44)	5 (27–69)
	c.1832G>T c.1833C>T	p.C611F	1	4	3/1	53 (36–64)	3/4 (75)	0	0	0	FMTC	0	0
	All		**12**	**50**	**30/20**	**42 (14–69)**	**36/50 (72)**	**5/50 (10)**	**1/44 (2)**	**0**	**MEN2A**	**6 (29–64)**	**7 (27–69)**
618	c.1852T>A	p.C618S	4	13	8/5	35 (18–59)	10/13 (77)	3/12 (25)	0	0	MEN2A	2 (42–59)	2 (31–39)
	c.1852T>C	p.C618R	16	28	22/6	35 (10–65)	28/28 (100)	11/28 (39)	0	1/12 (8)	MEN2A	11 (21–45)	7 (13–65)
	c.1852T>G	p.C618G	3	6	4/2	30 (21–57)	5/6 (83)	0	0	0	FMTC	0	1 (57)
	c.1853G>C	p.C618S	7	14	4/10	27 (9–63)	12/14 (86)	2/13 (15)	0	0	MEN2A	4 (9–50)	0
	c.1853G>A	p.C618Y	3	6	4/2	33 (25–55)	6/6 (100)	1/3 (33)	0	0	MEN2A	0	0
	c.1853G>T	p.C618F	4	27	9/18	45 (5–72)	21/27 (78)	2/21 (10)	2/21 (10)	0	MEN2A	3 (43–51)	3 (40–72)
	All		**37**	**94**	**51/43**	**35 (5–72)**	**82/94 (87)**	**19/84 (23)**	**2/78 (3)**	**1/56 (2)**	**MEN2A**	**20 (9–59)**	**13 (13–72)**
620	c.1858T>C	p.C620R	23	101	57/44	29 (6–73)	79/101 (78)	15/98 (15)	3/92 (3)	12/86 (14)	MEN2A, HSCR	27 (16–73)	20 (18–64)
	c.1858T>G	p.C620G	3	13	6/7	22	7/13 (54)	1/1 (100)	0	4/12 (33)	MEN2A	0	0
	c.1859G>A	p.C620Y	7	19	14/5	36 (18–76)	14/19 (74)	0	0	1/15 (7)	FMTC, HSCR	6 (31–60)	0
	c.1859G>C	p.C620S	4	8	6/2	40 (14–73)	14/20 (70)	3/20 (15)	0	0	MEN2A, HSCR	5 (28–73)	0
	c.1860C>G	p.C620W	1	2	2/0	40 (40)	1/2 (50)	0	0	0	FMTC	0	0
	c.1859G>T	p.C620F	3	8	7/1	35 (27–58)	3/8 (38)	2/7 (29)	0	0	MEN2A	1 (58)	0
	All		**41**	**151**	**92/59**	**31 (6–76)**	**111/151 (74)**	**19/142 (13)**	**3/133 (2)**	**17/130 (13)**	**MEN2A, HSCR**	**39 (16–73)**	**20 (18–64)**
All	**All**		**103**	**340**	**199/141**	**35 (4–86)**	**263/340 (77)**	**54/319 (17)**	**8/299 (3)**	**20/267 (7)**	**MEN2A, HSCR**	**72 (9–73)**	**41 (13–72)**

Table 2. Cases (Families) with Mutation per Exon

Center	Total no. of cases	Exon 609	Exon 611	Exon 618	Exon 620
Athens	27			4 (3)	23 (6)
Belgrade	5			2 (2)	3 (2)
Budapest	8	7 (2)	1 (1)		
Buenos Aires	16		6 (2)	6 (3)	4 (2)
Erlangen	2	2 (1)			
Frankfurt	10		10 (3)		
Freiburg	10			9 (2)	1 (1)
Girona	5	5 (1)			
Göttingen	24		24 (1)		
Groningen	20	1 (1)		16 (1)	3 (1)
Heidelberg	32	2 (1)		17 (11)	13 (8)
Innsbruck	1			1 (1)	
Leipzig	7	6 (1)			1 (1)
Mainz	8			8 (3)	
Nancy	3		2 (1)	1 (1)	
Nürnberg	1	1 (1)			
Padova	43	14 (1)		10 (4)	19 (2)
Porto Allegre	9			9 (3)	
Prague	13	2 (1)	2 (1)		9 (3)
Santiago de Chile	14			2 (1)	12 (2)
Sao Paolo	43		1 (1)		42 (3)
Utrecht	6		2 (1)	4 (2)	
Warsaw	30	5 (4)		4 (3)	21 (7)
Würzburg	3		2 (1)		1 (1)

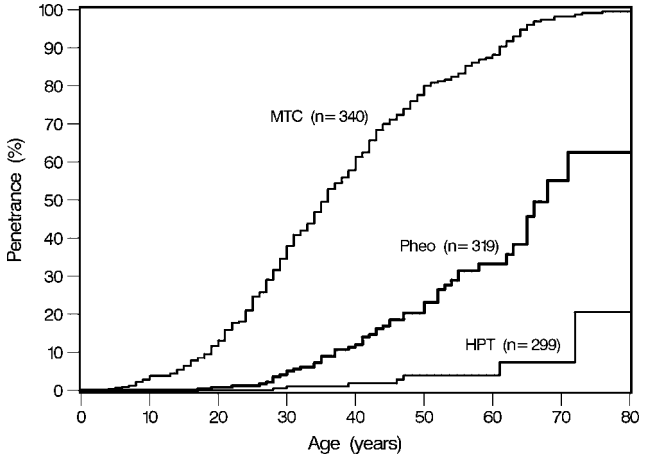

Figure 1. Penetrance of medullary thyroid carcinoma (MTC), pheochromocytoma (Pheo), and hyperparathyroidism (HPT) in 340 carriers of *RET* exon 10 germline mutations.

Table 3. Penetrance Data (%) for RET Codon 10 Mutations Compared to the C634W Mutation of Codon 11 Adjusted for C Cell Hyperplasia (CCH), MTC Stage T1–4N0M0, stage T1–4N1M0, and stage T1–4N0 or 1M1, and for Pheochromocytoma (pheo) and Hyperparathyroidism (HPT)

A. Penetrance for C cell hyperplasia						
All Exon 10 Codons	5.4	9.6	10.4	13.9	13.9	16.7
Codon 609	0	5.6	5.6	11.9	11.9	33.9
Codon 611	4.3	6.4	9.0	9.0	9.0	9.0
Codon 618	3.4	8.2	8.2	8.2	8.2	8.2
Codon 620	8.6	12.6	13.7	20.9	20.9	20.9
C634W	5.3	9.9	9.9	9.9	9.9	9.9
AGE→	10	20	30	40	50	60
B. Penetrance for MTC without metastases (T1–4N0M0)						
All Exon 10 Codons	2.9	10.2	26.6	42.0	57.0	67.3
Codon 609	2.4	11.2	27.0	46.6	69.2	74.3
Codon 611	0	7.1	15.6	34.0	44.3	59.4
Codon 618	1.2	8.7	31.1	43.7	60.2	72.8
Codon 620	5.3	12.0	27.0	42.2	54.6	61.6
C634W	6.8	26.0	36.1	49.6	65.9	76.1
AGE→	10	20	30	40	50	60
C. Penetrance for MTC with lymphnode metastases (T1–4N1M0)						
All Exon 10 Codons	0.3	2.3	11.0	25.4	43.2	51.3
Codon 609	0	0	3.4	19.8	34.4	45.3
Codon 611	0	0	3.2	6.6	24.6	24.6
Codon 618	1.2	2.5	8.8	20.4	41.5	52.5
Codon 620	0	3.8	17.9	38.9	55.0	64.0
C634W	0	0	16.3	26.2	41.6	41.6
AGE→	10	20	30	40	50	60
D. Penetrance for MTC with distant metastases (MTC T1–4N0or1M1)						
All Exon 10 Codons	0	0.8	5.1	11.4	18.4	27.4
Codon 609	0	0	0	0	0	0
Codon 611	0	0	2.9	7.0	7.0	23.9
Codon 618	0	1.2	4.3	11.8	20.2	28.1
Codon 620	0	0.9	8.2	16.5	29.3	35.7
C634W	0	0	5.6	8.8	8.8	8.8
AGE→	10	20	30	40	50	60
E. Penetrance for pheochromocytoma						
All Exon 10 Codons	0	0.8	5.1	12.0	23.1	33.2
Codon 609	0	2.8	5.7	9.4	26.9	47.0
Codon 611	0	0	0	3.1	15.4	15.4
Codon 618	0	0	5.1	13.5	24.8	42.5
Codon 620	0	0.9	6.9	16.0	22.9	26.3
C634W	0	1.4	19.6	42.6	67.1	70.1
AGE →	10	20	30	40	50	60
F. Penetrance for hyperparathyroidism						
All Exon 10 Codons	0	0	0.5	1.8	3.9	3.9
Codon 609	0	0	0	0	5.6	5.6
Codon 611	0	0	0	0	5.6	5.6
Codon 618	0	0	1.9	1.9	1.9	1.9
Codon 620	0	0	1.3	3.2	3.2	3.2
C634W	0	0	2.6	10.7	20.6	33.8
AGE →	10	20	30	40	50	60

(Table 1), although sample sizes per nucleotide change are small. In contrast, none of the 340 subjects had signs of MEN2B.

Age-Related Penetrance for *RET* Exon 10 Mutations

Age-related penetrance for each MEN2 component neoplasia was estimated for all the subjects as well as for each of the four cysteine codons. Overall for all exon 10 mutations, considering both symptomatic and screening ascertained cases, 50% penetrance was achieved by the age of 36 years for MTC, by 68 years for pheochromocytoma, and by 82 years for HPT (Fig. 1).

Age-related penetrance of MTC for mutations in codons 609, 611, 618, and 620 is shown in detail in Table 3. Fifty percent penetrance was achieved by the ages of 40, 44, 35, and 34 years for mutations in codons 609, 611, 618 and 620, respectively. Penetrance curves were significantly different among the four codons ($P = 0.018$, log-rank test; Fig. 2), and there was also a significant trend across the four codons ($P = 0.010$, Cox analysis), whereas within each of the affected codons, penetrance for MTC was not different for the given mutations (data not shown). The latter may be due to small sample sizes due to subset analysis.

We also compared age-related penetrance between symptomatic and screening ascertainment for MTC and found they were not different ($P = 0.90$). The ages at 50% penetrance for MTC was 36 years among those symptomatically ascertained and 35 years among those ascertained by screening. Similarly, the age-related penetrance at age 50 years was 83% and 76%, respectively.

For 319 subjects in whom pheochromocytoma was assessed, age-related penetrance at 50 years was 23% (Fig. 1 and Table 3). Penetrance curves were nearly different among codons ($P = 0.08$, log-rank test; Fig. 3), but there was no trend in pheochromocytoma penetrance across the four codons ($P = 0.99$, Cox analysis).

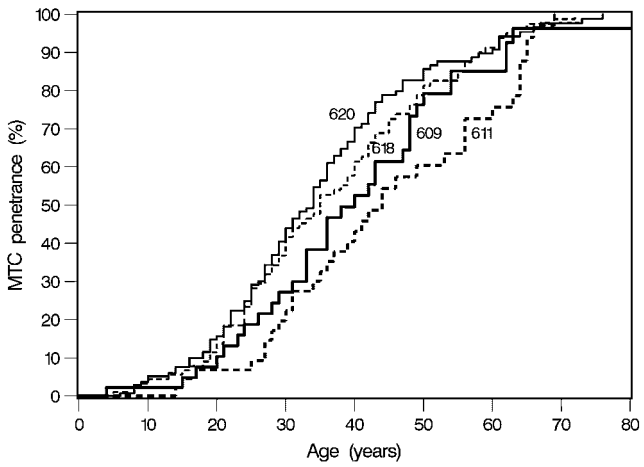

Figure 2. Penetrance of medullary thyroid carcinoma (MTC) of 340 carriers adjusted for germline mutations of the codons 609 ($n = 45$ carriers), 611 ($n = 50$ carriers), 618 ($n = 94$ carriers), and 620 ($n = 151$ carriers).

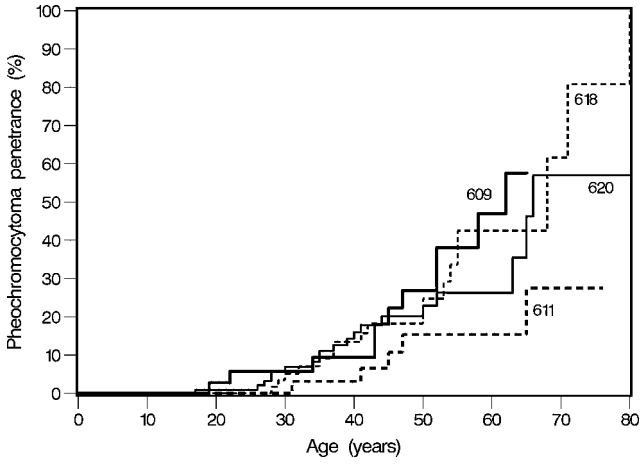

Figure 3. Penetrance of pheochromocytoma (Pheo) of 319 carriers adjusted for germline mutations of the codons 609 ($n = 43$ carriers), 611 ($n = 50$ carriers), 618 ($n = 84$ carriers), and 620 ($n = 142$ carriers).

Pheochromocytoma ascertainment was only known in 156 patients; however, penetrance was significantly different between pheochromocytoma ascertained from symptomatic assessment and by screening ($P < 0.001$; Fig. 4). Fifty percent penetrance was seen by 40 years for 29 symptomatic patients, and by 65 years for 127 screened patients, while penetrance by age 50 was 76% and 23%, respectively.

MTC Tumor Stage

TNM classification was recorded for 300 subjects. Age at diagnosis of any MTC is summarized by codon in Table 1. The youngest age at manifestation of MTC in these patient groups is 4, 14, 5, and 6 years for codons 609, 611, 618, and 620, respectively. The youngest age having lymph node metastasis was 21, 29, 9, and 16 years for the respective codons (Table 3). Penetrance for localized MTC was relatively high (67.3%) by the age of 60 for all exon 10 mutations with the highest penetrance for regional nodal involvement and distant metastases for codon 620 mutations

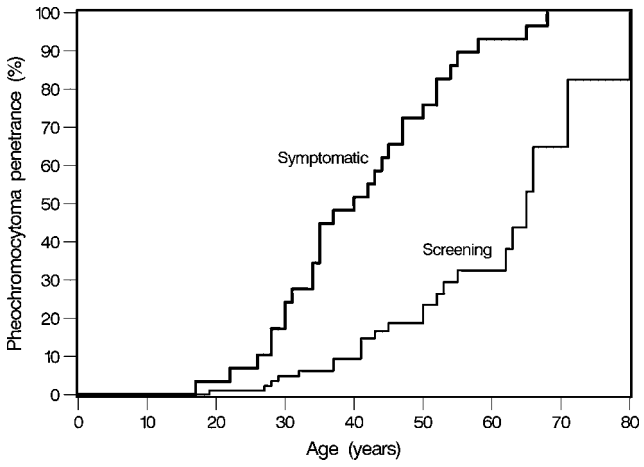

Figure 4. Penetrance of pheochromocytoma (Pheo) adjusted for detection by symtomatic pheochromocytoma (Symptomatic) and by screening-detected pheochromocytoma (Screening).

Table 4. Comparison of MTC Tumor Stage and Outcome in the Four Mutated Codons in Exon 10

	609 *n* (%)	611 *n* (%)	618 *n* (%)	620 *n* (%)	*P*-value
Normal histology	5/40 (12)	4/42 (10)	2/87 (2)	2/131 (2)	0.004[a]
					<0.001[b]
CCH only	4/40 (10)	4/42 (10)	7/87 (8)	22/131 (17)	
MTC: T1–4,N0,M0	23/40 (58)	21/42 (50)	45/87 (52)	48/131 (37)	
MTC: N1,M0	7/40 (18)	6/42 (14)	20/87 (23)	39/131 (30)	
MTC: N1,M1	1/40 (2)	7/42 (17)	13/87 (15)	20/131 (15)	
MTC: last info-cured[c]	26/32 (81)	25/34 (74)	39/72 (54)	51/104 (49)	0.002[a]
					<0.001[b]
MTC: metastasized[c]	4/33 (12)	8/33 (24)	24/76 (32)	43/104 (41)	0.011[a]
					0.002[b]

[a]Test of general association among codon groups.
[b]Test of trend across codons.
[c]Number of observations are different due to missing values.

(Table 3). Penetrance differed significantly among codons ($P = 0.005$, log-rank test), and there was also a trend across the four codons ($P = 0.003$, Cox analysis). In addition penetrance for the very early stage of C cell hyperplasia (CCH), as well for distant metastases (M1) are shown in Table 3. CCH penetrance was not different among codons ($P = 0.16$, log-rank test), nor was there a trend across codons ($P = 0.10$, Cox analysis). M1 penetrance was not quite different among codons ($P = 0.12$, log-rank test), but there was a trend across codons ($P = 0.020$, Cox analysis).

Comparing MTC stages, that is, metastasized or not metastasized and cured or not cured among codons using the chi-square test showed a significant difference among codons (Table 4). When ordered by codons, 609, 611, 618, and 620, higher stages ($P < 0.001$), increased the likelihood of metastasis ($P = 0.002$), and decreased the chance of cure ($P < 0.001$). Death caused by MTC was documented in 0, 1, 6, and 11 patients in the codons 609, 611, 618, and 620, respectively.

Gender, age, and mutated codon were examined as possible risk factors for MTC. In univariable chi-square analysis, female gender ($P = 0.008$) and older age ($P < 0.001$) were associated with higher risk of MTC. Specific codon mutated was not quite significant ($P = 0.06$); there appeared to be no trend across codons, but rather codon 618 mutation had the highest risk of MTC (76% MTC for codon 609, 72% for 611, 87% for 618, and 74% for 620).

In multivariable analysis, gender becomes nonsignificant, age remains significant ($P < 0.001$), and codon is not quite significant, but again, codon 618 has the highest risk of MTC ($P = 0.07$).

Discussion

Because the prevalence of *RET* exon 10 mutations, even all together, is much lower than that of the exon 11 codon 634 mutations, information on the natural history and age-related penetrance for component neoplasias remained scarce and was provided mostly by reports from single centers or on single large families [Calva et al., 2009; Mian et al., 2009; Moers et al., 1996; Siggelkow et al., 2001]. The American Thyroid Association (ATA) has, in particular, addressed this issue as a point of concern. Guidelines for diagnosis and management of MTC and other components of MEN2 have reached only a weak consensus level for these management guidelines for carriers of mutations in exon 10 [Kloos et al., 2009]. In this present study, we summarize and analyze comprehensive data for subjects carrying such *RET* exon 10 mutations based on a multicenter, multiethnic, and multinational series of 340 individuals who all have been registered using identical criteria. Thus, we are able to obtain molecular-based neoplasia risk profiles and codon-specific age-related penetrance on which to base genetic counseling and medical decision making.

Our current series demonstrates that 15 distinct mutations involving the four codons of *RET* exon 10 can all be associated with MEN2A. In contrast, the FMTC phenotype was seen to be associated with six distinct mutations, also involving all the four codons (Table 1). The studied number of carriers with these six mutations ranges from 2 to 26 (mean 11) per mutation. Therefore, the lack of pheochromocytoma in these subjects may be due to sample size and/or penetrance.

Missense mutations nearer the N-terminus are associated more with HSCR based on a clear mechanism [Santoro et al., 1999]. The first comprehensive genotype–phenotype study on MEN2 suggested that as high as one-third of all carriers of mutations in codons 618 and 620 had HSCR [Eng et al., 1996]. However, the sample sizes for exon 10 mutations in that series was relatively small. In even smaller series and single family reports, the prevalence of HSCR in codon 620 mutation carriers approached 50% [Butter et al., 2007]. In the present series, HSCR was only present in 20/267 informative carriers (7.5%), 2/37 (5.4%) in those with mutations in codon 609, 0/44 in codon 611, 1/56 (1.8%) in codon 618, and 17/130 (13.1%) in codon 620 (data not shown). This is a lower prevalence than initially predicted, however, it is tempting to speculate if other intra-*RET* modifying variants, whether in *cis* or *trans*, would modulate the HSCR phenotype in such individuals [Borrego et al., 1998, 1999, 2000, 2003; Fernandez et al., 2003].

Even before *RET* was identified, the classic penetrance of MTC in MEN2A is cited as 70% by age 70 by symptomatic presentation and 100% by age 70 by stimulated calcitonin screening [Ponder et al., 1988a,b]. Not surprisingly, age-related penetrance for codon 634 mutations approach $>85\%$ by age 80 for MTC and $>50\%$ by age 80 for pheochromocytoma [Iihara et al., 1997; Kloos et al., 2009; Milos et al., 2008; Modigliani et al., 1998]. Mutations in exon 10 were believed to have much lower age-related penetrances. Although our series showed that penetrance for MTC was 50% at age 36 for exon 10 mutations, which is lower than the 50% by age 28 for the C634W mutation [Milos et al., 2008], both exon 10 and 11 mutations reach $>80\%$ penetrance by age 50 and almost 100% by age 70. Interestingly, the penetrance for distant metastases from MTC was 9% for p.C634W compared to 36% for codon 620 mutations (Table 3). In contrast, the age-related penetrance of exon 10 mutations for pheochromocytomas was systematically lower than that for codon 634 mutations, such as for p.C634W (33 vs. 70% by age 60; Table 3). Notably, there is a significant difference in pheochromocytoma penetrance between those that were symptomatically ascertained compared to those ascertained by screening. This underscores the great importance of clinical screening for pheochromocytoma even in exon 10 mutation carriers, beginning in the late teens or early 20s. Our data support the ATA guidelines recommending initiation of screening for pheochromocytoma at the age of 20 in all mutations except those in codons 918 and 634, respectively; for these mutations, screening for pheochromocytoma is recommended beginning by the age of 8 years. Although sample size was small, the penetrance for HPT was 34% by age 60 for p.C634W compared to 3.9% by age 60 for exon 10 mutations (Table 3).

The results of this multicenter study of 340 patients carrying exon 10 mutations show differences in aggressiveness of MTC depending on the mutated codon. This results in earlier age at manifestation, more advanced tumor stage at manifestation, and decreased chance of cure when ordered by codons (620, 618, 611, and 609). In this patient group, age is an independent risk factor for the manifestation of MTC. Position of mutation was associated with metastatic disease, with the lowest likelihood among those with codon 609 mutations, and the highest, codon 620 mutations. This is important for the decisions regarding timing of premorbid diagnoses and treatment/prophylactic procedure in *RET* gene carriers, as age is an independent predictor of aggressive disease at thyroidectomy: MTC is more aggressive and might develop earlier in codons 618 and 620. This supports the clinical impression that patients with *RET* mutations in codons 609 and 611 are older at presentation [Calva et al., 2009; Hansen et al., 2000; Mian et al., 2009; Siggelkow et al., 2001], and have more indolent tumors than patients with *RET* mutations at 618 and 620 [Machens et al., 2009], but there are notable exceptions as shown in our systematic study. It is important to point out that also a patient with codons 609 mutation can present with MTC by age 4.

Multivariate analysis of our data reveals that age at presentation is associated with higher likelihood of metastases from MTC, a finding that is not surprising. What is also not surprising is that those who were ascertained symptomatically present at more advanced stages compared to those ascertained by screening, where a full one-third presents with normal histology or CCH only. Taken together, our observations strongly support clinical surveillance and/or timely prophylactic thyroidectomy even in exon 10 mutation carriers, which is certainly notated in the ATA MTC Practice Guidelines. However, the age to begin these remain unclear as the impression up until recently is exon 10 mutations do not normally confer early ages of onset of MTC. In our current series, however, the youngest age at diagnosis for MTC was 4 years (in a codon 609 mutation carrier) and for MTC with regional nodal metastases 9 years (in a codon 618 mutation carrier), respectively. Not surprisingly, therefore, those with codons 618 and 620 mutations had the highest prevalence of metastatic MTC, even higher than those with p.C634W. The youngest age for distant metastases amongst exon 10 mutation positive individuals was aged 13 years. The more typical age of MTC development for the exon 10 genotype reveals significant differences in aggressiveness of MTC manifestation, for example, higher penetrance of lymph node metastases and distant metastases in codons 618/620 in comparison to exon 609/611. The suggestion of the ATA guidelines to consider prophylactic surgery in patients carrying exon 10 codon 618 and 620 mutations at age of 5 years seems to be supported by our data. In patients with codon 609 and 611 prophylactic thyroidectomy possibly might be postponed under careful yearly clinical and laboratory examinations.

In summary, we have presented a large series of uniformly collected and registered cases with germline exon 10 mutations in *RET*, and have analyzed neoplastic risk profiles and age-related penetrance for each component neoplasia, which will help evidence-based genetic counseling and medical management.

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