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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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Complete List of Authors:	<p>Frank-Raue, Karin; Endocrine Practice and Molecular Laboratory Rybicki, Lisa; Cleveland Clinic, Department of Quantitative Health Sciences and Taussig Cancer Institute Erlic, Zoran; University of Freiburg Medical Centre, Section of Preventive Medicine, Department of Nephrology Schweizer, Heiko; University of Freiburg Medical Centre, Section of Preventive Medicine, Department of Nephrology Winter, Aurelia; University of Freiburg Medical Centre, Section of Preventive Medicine, Department of Nephrology Milos, Ioana; University of Freiburg Medical Centre, Section of Preventive Medicine, Department of Nephrology Toledo, Sergio; Hospital das Clinicas, University of Sao Paulo School of Medicine, Endocrine Genetics Unit (LIM-25), Endocrinology, Department of Internal Medicine Toledo, Rodrigo; Hospital das Clinicas, University of Sao Paulo School of Medicine, Endocrine Genetics Unit (LIM-25), Endocrinology, Department of Internal Medicine Tavares, Marcos; Hospital das Clinicas, University of Sao Paulo School of Medicine, Head and Neck Division, Department of Surgery Alevizaki, Maria; Alexandra Hospital, University School of Medicine, Endocrine Unit, Department of Medical Therapeutics Mian, Caterina; University of Padua, Operative Unit of Endocrinology Department of Surgical and Medical Sciences Siggelkow, Heide; University of Göttingen, Department of Endocrinology Hübner, Michael; University of Göttingen, Department of Endocrinology Wohllk, Nelson; Universidad de Chile, Endocrine Section, Hospital del Salvador Opocher, Giuseppe; University of Padova, Padova, Familial Cancer Clinic, Veneto Institute of Oncology, IRCCS & Department of Medical and Surgical Sciences Dvořáková, Šárka; Institute of Endocrinology Prague, Department</p>

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3	
4	of Molecular Endocrinology
5	Bendlova, Bela; Maria Sklodowska Curie Memorial Center and
6	Institute of Oncology, Department of Nuclear Medicine and
7	Oncological Endocrinology
8	Czertwertynska, Małgorzata; Maria Sklodowska Curie Memorial
9	Center and Institute of Oncology, Department of Endocrinology
10	Skasko, Elżbieta; Maria Sklodowska Curie Memorial Center and
11	Institute of Oncology, Department of Endocrinology
12	Barontini, Marta; Hospital de Niños R. Gutiérrez, Centro de
13	Investigaciones Endocrinológicas
14	Sanso, Gabriela; Hospital de Niños R. Gutiérrez, Centro de
15	Investigaciones Endocrinológicas
16	Vorländer, Christian; Bürgerhospita, Department of Endocrine
17	Surgery
18	Maia, Ana; Hospital de Clínicas de Porto Alegre, Universidade
19	Federal do Rio Grande do Sul, Thyroid Section, Endocrine Division
20	Patocs, Attila; Semmelweis University, Molecular Medicine Research
21	Group, Hungarian Academy of Sciences and 2nd Department of
22	Medicine
23	Links, Thera; University Medical Center Groningen, Department of
24	Endocrinology
25	de Groot, Jan; University Medical Center Groningen, Department of
26	Endocrinology
27	Kerstens, Michiel; University Medical Center Groningen, Department
28	of Endocrinology
29	Valk, Gerlof D.; University Medical Center Utrecht, Department of
30	Internal Medicine
31	Miehle, Konstanze; Universityhospital of Leipzig, Department of
32	Endocrinology
33	Musholt, Thomas; University Medical Center of the Johannes
34	Gutenberg University Mainz, Endocrine Surgery, Department of
35	General and Abdominal Surgery
36	Biarnes, Josefina; Hospital Dr Josep Trueta de Girona,
37	Endocrinology Unit
38	Damjanovic, Svetozar; University of Belgrade, Institute of
39	Endocrinology, Diabetes & Metabolic Diseases, Medical School
40	Muresan, Mihaela; Hôpital N.-D. de Bon Secours, Department of
41	Endocrinology
42	Wüster, Christian; Private Praxis for Endocrinology
43	Fassnacht, Martin; University Hospital, Department of Medicine I
44	Peczowska, Mariola; Institute of Cardiology, Department of
45	Hypertension
46	Fauth, Christine; Innsbruck Medical University, Division of Clinical
47	Genetics, Department of Medical Genetics, Molecular and and
48	Clinical Pharmacology
49	Golcher, Henriette; Friedrich-Alexander Universityhospital of
50	Erlangen, Department of Surgery
51	Walter, Martin; Institute of Nuclear Medicine, University Hospital
52	Pichl, Josef; St. Theresien-Hospital
53	Raue, Friedhelm; Endocrine Practice and Molecular Laboratory
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Risk profiles and penetrance estimations in multiple endocrine neoplasia type

2A caused by germline *RET* mutations located in exon 10

Karin Frank-Raue^{1*}, Lisa A. Rybicki^{2*}, Zoran Erlic^{3*}, Heiko Schweizer^{3*}, Aurelia Winter³, Ioana Milos^{3,4}, Sergio PA Toledo⁵, Rodrigo A Toledo⁵, Marcos R Tavares⁶, Maria Alevizaki⁷, Caterina Mian⁸, Heide Siggelkow⁹, Michael Hüfner¹⁰, Nelson Wohllk¹¹, Giuseppe Opocher¹², Šárka Dvořáková¹³, Bela Bendlova¹⁴, Małgorzata Czetwertynska¹⁵, Elżbieta Skasko¹⁵, Marta Barontini¹⁶, Gabriela Sanso¹⁶, Christian Vorländer¹⁷, Ana Luiza Maia¹⁸, Attila Patocs¹⁹, Thera P. Links²⁰, Jan Willem de Groot²⁰, Michiel N. Kerstens²⁰, Gerlof D Valk²¹, Konstanze Miehle²², Thomas J. Musholt²³, Josefina Biarnes²⁴, Svetozar Damjanovic²⁵, Mihaela Muresan²⁶, Christian Wüster²⁷, Martin Fassnacht²⁸, Mariola Peczkowska²⁹, Christine Fauth³⁰, Henriette Golcher³¹, Martin A Walter³², Josef Pichl³³, Friedhelm Raue^{1**}, Charis Eng^{34**}, Hartmut PH Neumann^{3**} for the International *RET* Exon 10 Consortium

*These authors contributed equally to this study.

** These authors are senior authors.

¹Endocrine Practice and Molecular Laboratory, Heidelberg, Germany

²Department of Quantitative Health Sciences and Taussig Cancer Institute, Cleveland Clinic; Cleveland, OH, 44195; USA

³Section of Preventive Medicine, Department of Nephrology, University of Freiburg

1
2 Medical Centre, Freiburg im Breisgau, Germany
3

4
5 ⁴Department of Endocrinology of the University of Medicine and Pharmacy “Victor
6 Babes” Timisoara, Romania
7

8
9
10 ⁵ Endocrine Genetics Unit (LIM-25), Endocrinology, Department of Internal Medicine,
11 Hospital das Clinicas, University of Sao Paulo School of Medicine, Sao Paulo, Brazil
12

13
14 ⁶Head and Neck Division, Department of Surgery, Hospital das Clinicas, University of
15 Sao Paulo School of Medicine, Sao Paulo, Brazil
16

17
18 ⁷Endocrine Unit, Department of Medical Therapeutics, Alexandra Hospital,
19 University School of Medicine, Athens, Greece
20

21
22 ⁸Operative Unit of Endocrinology Department of Surgical and Medical Sciences
23 University of Padua, Italy
24

25
26
27 ⁹Department of Gastroenterology and Endocrinology, University of Göttingen and
28 Endokrinologikum Göttingen, Germany
29

30
31 ¹⁰Endokrinologikum Göttingen, Germany
32

33
34
35 ¹¹Endocrine Section, Hospital del Salvador, Universidad de Chile, Santiago de Chile,
36 Chile
37

38
39
40 ¹²Familial Cancer Clinic, Veneto Institute of Oncology, IRCCS & Department of
41 Medical and Surgical Sciences, University of Padova, Padova, Italy
42

43
44
45 ¹³Department of Molecular Endocrinology of the Institute of Endocrinology Prague,
46 Prague, Czech Republic
47

48
49
50 ¹⁴Department of Nuclear Medicine and Oncological Endocrinology, Maria
51 Sklodowska Curie Memorial Center and Institute of Oncology, Warsaw, Poland
52

1
2 ¹⁵Department of Endocrinology, Maria Sklodowska Curie Memorial Center and
3
4 Institute of Oncology, Warsaw, Poland

5
6 ¹⁶Centro de Investigaciones Endocrinológicas, Hospital de Niños R. Gutiérrez,
7
8 Buenos Aires, Argentina

9
10
11 ¹⁷Department of Endocrine Surgery, Bürgerhospital, Frankfurt, Germany

12
13
14 ¹⁸Thyroid Section, Endocrine Division Hospital de Clínicas de Porto Alegre,
15
16 Universidade Federal do Rio Grande do Sul, Brazil

17
18
19 ¹⁹Molecular Medicine Research Group, Hungarian Academy of Sciences and 2nd
20
21 Department of Medicine, Semmelweis University, Budapest, Hungary

22
23 ²⁰University Medical Center Groningen, Department of Endocrinology, Groningen,
24
25 The Netherlands

26
27 ²¹Department of Internal Medicine, University Medical Center Utrecht, The
28
29 Netherlands

30
31
32 ²²Department of Endocrinology, Universityhospital of Leipzig, Leipzig, Germany

33
34
35 ²³Endocrine Surgery, Department of General and Abdominal Surgery, University
36
37 Medical Center of the Johannes Gutenberg University Mainz, Mainz, Germany

38
39 ²⁴Endocrinology Unit, Hospital Dr Josep Trueta de Girona, Girona, Spain

40
41 ²⁵Institute of Endocrinology, Diabetes & Metabolic Diseases, Medical School,
42
43 University of Belgrade, Serbia

44
45
46 ²⁶Department of Endocrinology, Hôpital N.-D. de Bon Secours, Metz, France

47
48
49 ²⁷Private Praxis for Endocrinology, Mainz, Germany

50
51 ²⁸Department of Medicine I, University Hospital, University of Würzburg, Germany

1
2 ²⁹Department of Hypertension, Institute of Cardiology, Warsaw, Poland
3

4 ³⁰Division of Clinical Genetics, Department of Medical Genetics, Molecular and
5 and
6 Clinical Pharmacology, Innsbruck Medical University, Innsbruck, Austria
7

8
9 ³¹Department of Surgery, Friedrich-Alexander Universityhospital of Erlangen,
10
11 Erlangen, Germany
12

13
14 ³²Institute of Nuclear Medicine, University Hospital, Basel, Switzerland
15

16
17 ³³St. Theresien-Hospital, Nuernberg, Germany
18

19
20 ³⁴Genomic Medicine Institute, Lerner Research Institute, Taussig Cancer Institute,
21
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23
24
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Short Title: MEN 2 in Patients with RET Exon 10 Mutations

Key words: Multiple endocrine neoplasia type 2, medullary thyroid carcinoma, pheochromocytoma, genotype-phenotype correlation

Corresponding address:

Hartmut P.H. Neumann, MD

Medizinische Universitätsklinik,

Abteilung Innere Medizin 4, Sektion für Präventive Medizin

Hugstetter Str. 55

D 79106 Freiburg, Germany

Phone: 0761 270 3578, Fax: 0761 270 3778

Email: hartmut.neumann@uniklinik-freiburg.de

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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 103 families, age 2-86, 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 amongst 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 pre-morbid diagnosis and personalized treatment/prophylactic procedure decisions depending on specific *RET* exon 10 codons affected.

Word count: 194

Introduction

Multiple endocrine neoplasia type 2 (MEN 2) is an autosomal dominant multi-glandular tumor syndrome, affecting derivatives from neural crest. MEN 2 is caused by germline mutations of the *RET* proto-oncogene (OMIM164761), 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 C, 1996 ; Zbuk and Eng, 2007). When *RET* mutations were found in the large majority of MEN 2 patients and a *RET* genotype-clinical phenotype found (Eng C, 1996), this was the paradigm for the practice of clinical cancer genetics (Kloos, et al., 2009; Zbuk and Eng, 2007). Accurate molecular diagnosis, pre-morbid predictive testing and gene-informed medical management became possible.

Deleted: (Kloos, et al., 2009; Zbuk and Eng, 2007; Eng Ann NY Acad Sci 2010 in press)

Based on combinations of clinical manifestations, MEN 2 is historically classified into three clinical subtypes, MEN 2A (MIM# 171400), MEN 2B (MIM# 162300), and familial medullary thyroid carcinoma (FMTC, MIM# 155240). The components of MEN 2A are medullary thyroid carcinoma (MTC), pheochromocytoma and parathyroid adenoma and/or hyperplasia (HPT). In MEN 2B, 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). MEN 2B is considered more aggressive than MEN 2A or FMTC, because the age-of-onset of the neoplasias is a mean 10 years earlier than that of MEN 2A, and metastases from MTC occur as early as 3 years of age (Kloos, et al., 2009).

The spectrum of germline *RET* mutations in MEN 2 are encompassed by 64 distinct mutations that lie in 32 codons belonging to 8 exons (Kloos, et al., 2009) . It is clear that the MEN 2B-defining mutations, M918T and A883F, portend an aggressive

1
2 course, with management appropriately aggressive and pre-emptive (Kloos, et al.,
3
4 2009). It is also obvious and relatively consistent that the exon 11 C634R, the most
5
6 common mutation seen in MEN 2A, predicts for the development of MTC,
7
8 pheochromocytoma and HPT, at relatively young ages, and so management is
9
10 tailored accordingly (Eng C, 1996 ; Frank-Raue, et al., 2006; Kloos, et al., 2009;
11
12 Neumann, et al., 2002). However, apart from vague generalizations such as non-634
13
14 mutations are not as aggressive, perhaps have later ages of onset and decreased
15
16 penetrance, the breadth of clinical presentations and course, and the less-than-firm
17
18 data preclude clinicians from altering medical management based on precise single-
19
20 codon/specific missense mutation genotype. For instance, studies have shown that
21
22 mutations at codon 634, the most frequently mutated in MEN 2, result in a receptor
23
24 tyrosine kinase that is constitutively active by the tendency of mutant *RET* monomers
25
26 to undergo dimerization, a situation that simulates ligand binding. Whereas cysteine
27
28 residues in wild-type *RET* form intramolecular disulfide bonds, mutation of a cysteine
29
30 residue leaves an unpaired residue. Unpaired cysteines from two mutant *RET*
31
32 monomers can dimerize by formation of a disulfide bond. Taking into account the
33
34 clinical presentation, we know that the C634R mutation is highly penetrant and
35
36 associated with the more severe forms of MEN 2A (Eng C, 1996 ; Kloos, et al.,
37
38 2009). Similarly, while investigating pheochromocytoma presentations in our
39
40 Registry, we noted that MTC does not always occur before other component
41
42 neoplasias in those with C634W mutations (Neumann, et al., 2007). Therefore, we
43
44 believe that single nucleotide genotype-specific clinical risk profiles comprising risk of
45
46 developing each component neoplasia, the ages-at-onset, penetrance and clinical
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48 course might be useful for tailoring more precise clinical care.

49
50 In contrast to well-defined risk profiles for carriers of the C634R, C634Y, C634W,
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1
2 A883F, and M918T mutations, mutations located in the codons of *RET* exon 10 are
3 relatively rare (Mian, et al., 2009; Moers, et al., 1996; Siggelkow, et al., 2001), and
4 their anecdotal penetrance and ages-of-onset have resulted in appropriately cautious
5 and tentative non-consensual clinical guidelines (Brandi, et al., 2001; Kloos, et al.,
6 2009). Here, concerted effort from an international consortium have resulted in *RET*
7 exon 10 genotyping, documented phenotypes-at-presentation and longitudinal
8 clinical data with the purpose of generating codon- and nucleotide-specific genotype-
9 adjusted neoplasia risk profiles for exon 10 mutations.
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24 **Patients and Methods**

26 **Patients**

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29 The inclusion criterion for registrants was a proven carrier status of a germline
30 mutation in exon 10 of the *RET* gene. In addition, relatives of index registrants were
31 also registered if an MTC or a pheochromocytoma was diagnosed histologically. The
32 ascertainment of carriers of such mutations was performed in 3 steps. First, *RET*
33 exon 10 mutation carriers were identified from existing registrants in the European-
34 American Pheochromocytoma Registry (EAPR), which has been previously
35 described (Erlic, et al., 2009; Neumann, et al., 2002; Neumann, et al., 2004;
36 Neumann, et al., 2007; Schiavi, et al., 2005). Second, all clinicians and researchers
37 who had contributed to this registry or to our previous risk estimation study of *RET*
38 C634W carriers were contacted (Milos, et al., 2008). Finally, all known centers with
39 clinical activities in MEN 2 or one of the component neoplasias (eg, MTC or
40 pheochromocytoma) were asked to contribute any relevant data to this study.
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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 MEN 2, as follows.

For all three components, i.e., 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-hour 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 MEN 2 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

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2 classification (DeLellis RA, 2004; Greene LF, 2002; Sobin LH, 2002). T1N0M0 for
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4 MTC < 2cm in diameter, T2N0M0 for MTC of 2-4cm in diameter, T3 > 4cm either
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6 limited to the thyroid or with minimal invasion to the sternocleidomastoid muscle or
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8 perithyroidal soft tissue, T4a any size with invasion to subcutaneous tissue, larynx,
9
10 trachea, esophagus or recurrent laryngeal nerve, T4b invasion of prevertebral fascia,
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12 carotid artery or mediastinal vessels, N1 for lymph node metastases and M1 for
13
14 distant metastases. In cases of persistent or clearly elevated calcitonin levels after
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16 thyroidectomy including regional lymph node dissection, we classified 6 patients with
17
18 distant metastases, although radiological evidence was not present.

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20
21 This study has been approved by the respective institutional review boards for
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23 human subjects' protection in accordance with the ethical standards of each country
24
25 and center.

26 27 28 29 30 **Statistical Analysis**

31
32
33 Categorical variables were summarized as frequency counts and percentages.
34
35 Continuous variables were summarized as the median and range. MTC stage,
36
37 cure/no-cure, and presence/absence of metastasis were compared among codons.
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39 Comparisons were made with the Chi-square test which determines, if variables differ
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41 between at least two of the four codons; and with the Cochran-Mantel-Haenszel
42
43 mean score test (TNM stage) or Cochran-Armitage trend test (cure, metastasis).
44
45 These trend tests account for the ordering of codons by distance from the
46
47 transmembrane domain and assess trends in stage, cure or metastasis. Risk factors
48
49 for MTC were assessed via Chi-square tests and logistic regression analysis. Three
50
51 candidate MTC risk factors were assessed: gender, age, and codon. All three

1
2 variables were included in a multivariate logistic regression model whether or not
3
4 they were statistically significant. Age-dependent penetrance estimates of MTC,
5
6 pheochromocytoma and HPT were calculated using the Kaplan-Meier method.
7
8 Penetrance estimates were compared among codons using the log-rank test and with
9
10 Cox proportional hazards analysis. The log-rank analysis assesses penetrance
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12 differences among codons, while the Cox analysis assesses trends across codons.
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14 Some patients had incomplete data regarding the different manifestations and were
15
16 excluded from penetrance estimates. Thus, the penetrance data for MTC are based
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18 on 340 subjects, pheochromocytoma 319 subjects, and HPT 299 subjects. All
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20 analyses were done using SAS® software (SAS® Institute, Inc., Cary, NC, USA). All
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22 statistical tests were two-sided, and $P \leq 0.05$ was used to indicate statistical
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24 significance.
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32 Results

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34 Overall, 340 individuals, 199 females and 141 males, had germline mutations
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36 in exon 10 of the *RET* gene and their clinical phenotypes and penetrance were
37
38 analyzed for purposes of this study. The mutations affected only cysteine codons
39
40 609, 611, 618 and 620 (Table 1). The 340 subjects, 255 European and 85 South
41
42 American (comprising 280 of Caucasian, 20 of Hispanic, 3 of Italian-German-
43
44 Hispanic, 37 of Indian-Caucasian and 1 of Gypsy origin), belonged to 103 different
45
46 families and hailed from 14 different countries (Table 1). Each of the centers
47
48 contributed from 1 to 43 cases. The center specific distributions of cases amongst the
49
50 codons is shown in Table 2.

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2 Codon 609 mutations were found in 45 subjects from 13 families. Codon 611
3
4 mutations were noted in 50 subjects from 12 families. Codon 618 mutations were
5
6 found in 94 subjects from 37 families. Codon 620 mutations were identified in 151
7
8 subjects from 41 families (Table 1).
9

10 11 **Phenotypic Spectrum of *RET* Exon 10 Mutations**

12
13 MTC was the most common neoplasia, with 263 affected (77%). In the
14
15 remaining 23%, diagnosis of MTC was excluded by objective means. The median
16
17 age at diagnosis of MTC was 35 (range 4-86). Of note, symptomatic ascertainment of
18
19 MTC occurred in 47% of the 263 patients whereas in 53%, their MTC was detected
20
21 by screening.
22

23
24 Pheochromocytoma was documented in 54 of 319 subjects (17%). The
25
26 median age at diagnosis of pheochromocytoma was 42 years (17-80).
27
28 Pheochromocytoma was observed in individuals with mutations of all four codons.
29
30 However, pheochromocytoma was not observed in carriers of 7 of the 22 distinct
31
32 mutations. The frequencies of pheochromocytoma varied, occurring in 26% of those
33
34 patients with codon 609 mutations, 10% codon 611, 23% codon 618 and 13% codon
35
36 620 (Table 1). All pheochromocytomas were adrenal in location. Bilateral
37
38 pheochromocytoma was present in 15 of 54 (28%) affected subjects. None had
39
40 malignant pheochromocytoma. Symptomatic pheochromocytoma occurred in 29
41
42 (54%) of the 54 patients; 24 of these 29 also had MTC. Among these 24,
43
44 pheochromocytoma was diagnosed at least one year prior to MTC in 6 patients
45
46 (25%), within 1 year of MTC in 8 (33%), and at least one year after MTC in the
47
48 remaining 10 (42%).

49
50 HPT was present in 8 of 299 subjects (2.7%). Median age at diagnosis of HPT
51
52 was 46 years (range 28-82). Of the 8 with HPT, 7 were ascertained by screening at

1
2 an asymptomatic stage. Although sample size is small, HPT was found in 4 out of 22
3
4 distinct mutations residing in all four exon 10 codons.

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6 Regarding codon-specific neoplasias seen in our MEN 2 series, MTC and
7
8 pheochromocytoma were present in carriers of germline mutations of each of the four
9
10 codons. Notably, codon 609 mutation carriers as a group have similar frequencies of
11
12 MTC and pheochromocytoma as the groups carrying mutations in codons 611, 618
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14 and 620 ($p=0.2$, Fisher 2-tailed exact test). However, if we analyzed the frequencies
15
16 of MTC and pheochromocytoma for each distinct mutation, FMTC was found in
17
18 patients carrying 6 specific missense mutations distributed across all 4 codons (Table
19
20 1), although sample sizes per nucleotide change are small. In contrast, none of the
21
22 340 subjects had signs of MEN 2B.

23 24 25 **Age-related Penetrance for *RET* Exon 10 Mutations**

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27 Age-related penetrance for each MEN 2 component neoplasia was estimated
28
29 for all the subjects as well as for each of the 4 cysteine codons. Overall for all exon
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31 10 mutations, considering both symptomatic and screening ascertained cases, 50%
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33 penetrance was achieved by the age of 36 years for MTC, by 68 years for
34
35 pheochromocytoma, and by 82 years for HPT (Fig. 1).

36
37 **Age-related penetrance of MTC for mutations in codons 609, 611, 618, and**
38
39 **620 is shown in detail in table 3.** Fifty % penetrance was achieved by the ages of 40,
40
41 44, 35, and 34 years for mutations in codons 609, 611, 618 and 620, respectively.
42
43 Penetrance curves were significantly different among the four codons ($p=0.018$, log-
44
45 rank test; Fig. 2), and there was also a significant trend across the four codons
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47 ($p=0.010$, Cox analysis) whereas within each of the affected codons, penetrance for
48
49 MTC was not different for the given mutations (data not shown). The latter may be
50
51 due to small sample sizes due to subset analysis.

1
2 We also compared age-related penetrance between symptomatic and
3 screening ascertainment for MTC and found they were not different ($p=0.90$). The
4 ages at 50% penetrance for MTC was 36 years among those symptomatically
5 ascertained and 35 years among those ascertained by screening. Similarly, the age-
6 related penetrance at age 50 years was 83% and 76%, respectively.
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10 For 319 subjects in whom pheochromocytoma was assessed, age-related
11 penetrance at 50 years was 23% (Fig. 1 and Table 3). Penetrance curves were
12 nearly different among codons ($p=0.08$, log-rank test; Fig. 3), but there was no trend
13 in pheochromocytoma penetrance across the four codons ($p=0.99$, Cox analysis).
14 Pheochromocytoma ascertainment was only known in 156 patients; however,
15 penetrance was significantly different between pheochromocytoma ascertained from
16 symptomatic assessment and by screening ($p<0.001$; Fig. 4). Fifty % penetrance was
17 seen by 40 years for 29 symptomatic patients, and by 65 years for 127 screened
18 patients, while penetrance by age 50 was 76% and 23%, respectively.
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31 MTC Tumor Stage

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33 TNM classification was recorded for 300 subjects. Age at diagnosis of any
34 MTC is summarized by codon in table 1. The youngest age at manifestation of MTC
35 in these patient groups is 4, 14, 5, and 6 years for codons 609, 611, 618, and 620,
36 respectively. The youngest age having lymph node metastasis was 21, 29, 9, and 16
37 years for the respective codons (Table 3). Penetrance for localized MTC was
38 relatively high (67.3%) by the age of 60 for all exon 10 mutations with the highest
39 penetrance for regional nodal involvement and distant metastases for codon 620
40 mutations (Table 3). Penetrance differed significantly among codons ($p=0.005$, log-
41 rank test), and there was also a trend across the four codons ($p=0.003$, Cox
42 analysis). In addition penetrance for the very early stage of C cell hyperplasia (CCH),
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2 as well for distant metastases (M1) are shown in table 3. CCH penetrance was not
3 different among codons (p=0.16, log-rank test), nor was there a trend across codons
4 (p=0.10, Cox analysis). M1 penetrance was not quite different among codons
5 (p=0.12, log-rank test), but there was a trend across codons (p=0.020, Cox analysis).
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10 [Yes, this is fine to be added here as well]

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12 Comparing MTC stages, i.e. metastasized or not metastasized and cured or
13 not cured among codons using the Chi-square test showed a significant difference
14 among codons (Table 4). When ordered by codons, 609, 611, 618 and 620, higher
15 stages (p<0.001), increased the likelihood of metastasis (p=0.002), and decreased
16 the chance of cure (p<0.001). Death caused by MTC was documented in 0, 1, 6, and
17 11 patients in the codons 609, 611, 618, and 620, respectively.

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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 non-significant, 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

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2 American Thyroid Association (ATA) has, in particular, addressed this issue as a
3 point of concern. Guidelines for diagnosis and management of MTC and other
4 components of MEN 2 have reached only a weak consensus level for these
5 management guidelines for carriers of mutations in exon 10 (Kloos, et al., 2009). In
6 this present study, we summarize and analyze comprehensive data for subjects
7 carrying such *RET* exon 10 mutations based on a multicenter, multiethnic and
8 multinational series of 340 individuals who all have been registered using identical
9 criteria. Thus, we are able to obtain molecular-based neoplasia risk profiles and
10 codon-specific age-related penetrance on which to base genetic counseling and
11 medical decision-making.
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14 Our current series demonstrates that 15 distinct mutations involving the 4 codons of
15 *RET* exon 10 can all be associated with MEN 2A. In contrast, the FMTC phenotype
16 was seen to be associated with 6 distinct mutations, also involving all the 4 codons
17 (Table 1). The studied number of carriers with these 7 mutations ranges from 2 to 26
18 (mean 11) per mutation. Therefore, the lack of pheochromocytoma in these subjects
19 may be due to sample size and/or penetrance.
20

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22 Missense mutations nearer the N-terminus are associated more with HSCR
23 based on a clear mechanism (Santoro, et al., 1999). The first comprehensive
24 genotype-phenotype study on MEN 2 suggested that as high as 1/3 of all carriers of
25 mutations in codons 618 and 620 had HSCR (Eng C, 1996). However, the sample
26 sizes for exon 10 mutations in that series was relatively small. In even smaller series
27 and single family reports, the prevalence of HSCR in codon 620 mutation carriers
28 approached 50% (Butter, et al., 2007). In the present series, HSCR was only present
29 in 20/267 informative carriers (7.5%), 2/37 (5.4%) in those with mutations in codon
30 609, 0/44 in codon 611, 1/56 (1.8%) in codon 618, and 17/130 (13.1%) in codon 620
31 (data not shown). This is a lower prevalence than initially predicted, however, it is
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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; Borrego, et al., 2000; Borrego, et al., 1999; Borrego, et al., 2003; Fernandez, et al., 2003).

Even before *RET* was identified, the classic penetrance of MTC in MEN 2A is cited as 70% by age 70 by symptomatic presentation and 100% by age 70 by stimulated calcitonin screening (Ponder, et al., 1988a; Ponder, et al., 1988b). 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. While 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

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2 penetrance for HPT was 34% by age 60 for p.C634W compared to 3.9% by age 60
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4 for exon 10 mutations (Table 3).
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6 The results of this multicenter study of 340 patients carrying exon 10
7 mutations show differences in aggressiveness of MTC depending on the mutated
8 codon. This results in earlier age at manifestation, more advanced tumor stage at
9 manifestation and decreased chance of cure when ordered by codons (620, 618,
10 611, and 609). In this patient group, age is an independent risk factor for the
11 manifestation of MTC. Position of mutation was associated with metastatic disease,
12 with the lowest likelihood amongst those with codon 609 mutations, and the highest,
13 codon 620 mutations. This is important for the decisions regarding timing of
14 premorbid diagnoses and treatment/prophylactic procedure in *RET* gene carriers, as
15 age is an independent predictor of aggressive disease at thyroidectomy: MTC is
16 more aggressive and might develop earlier in codons 618 and 620. This supports the
17 clinical impression that patients with *RET* mutations in codons 609 and 611 are older
18 at presentation (Calva, et al., 2009; Hansen, et al., 2000; Mian, et al., 2009;
19 Siggelkow, et al., 2001), and have more indolent tumors than patients with *RET*
20 mutations at 618 and 620 (Machens, et al., 2009), but there are notable exceptions
21 as shown in our systematic study. It is important to point out that also a patient with
22 codons 609 mutation can present with MTC by age 4.
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39 Multivariate analysis of our data reveals that age at presentation is associated
40 with higher likelihood of metastases from MTC, a finding that is not surprising. What
41 is also not surprising is that those who were ascertained symptomatically present at
42 more advanced stages compared to those ascertained by screening, where a full 1/3
43 presents with normal histology or CCH only. Taken together, our observations
44 strongly support clinical surveillance and/or timely prophylactic thyroidectomy even in
45 exon 10 mutation carriers, which is certainly notated in the ATA MTC Practice
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2 Guidelines. However, the age to begin these remain unclear as the impression up
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4 until recently is exon 10 mutations do not normally confer early ages of onset of MTC.
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6 In our current series, however, the youngest age at diagnosis for MTC was 4 years
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8 (in a codon 609 mutation carrier) and for MTC with regional nodal metastases 9
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10 years (in a codon 618 mutation carrier), respectively. Not surprisingly, therefore,
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12 those with codons 618 and 620 mutations had the highest prevalence of metastatic
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14 MTC, even higher than those with p.C634W. The youngest age for distant
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16 metastases amongst exon 10 mutation positive individuals was aged 13 years. The
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18 more typical age of MTC development for the exon 10 genotype reveals significant
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20 differences in aggressiveness of MTC manifestation e.g. higher penetrance of lymph
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22 node metastases and distant metastases in codons 618/620 in comparison to exon
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24 609/611. The suggestion of the ATA guidelines to consider prophylactic surgery in
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26 patients carrying exon 10 codon 618 and 620 mutations at age of 5 years seems to
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28 be supported by our data. In patients with codon 609 and 611 prophylactic
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30 thyroidectomy possibly might be postponed under careful yearly clinical and
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32 laboratory examinations.

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34 In summary, we have presented a large series of uniformly collected and
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36 registered cases with germline exon 10 mutations in *RET*, and have analyzed
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38 neoplastic risk profiles and age-related penetrance for each component neoplasia,
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40 which will help evidence-based genetic counseling and medical management.
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43 **Word count: 3844**

44 45 46 47 48 49 50 **References**

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Figure Legends

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

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).

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

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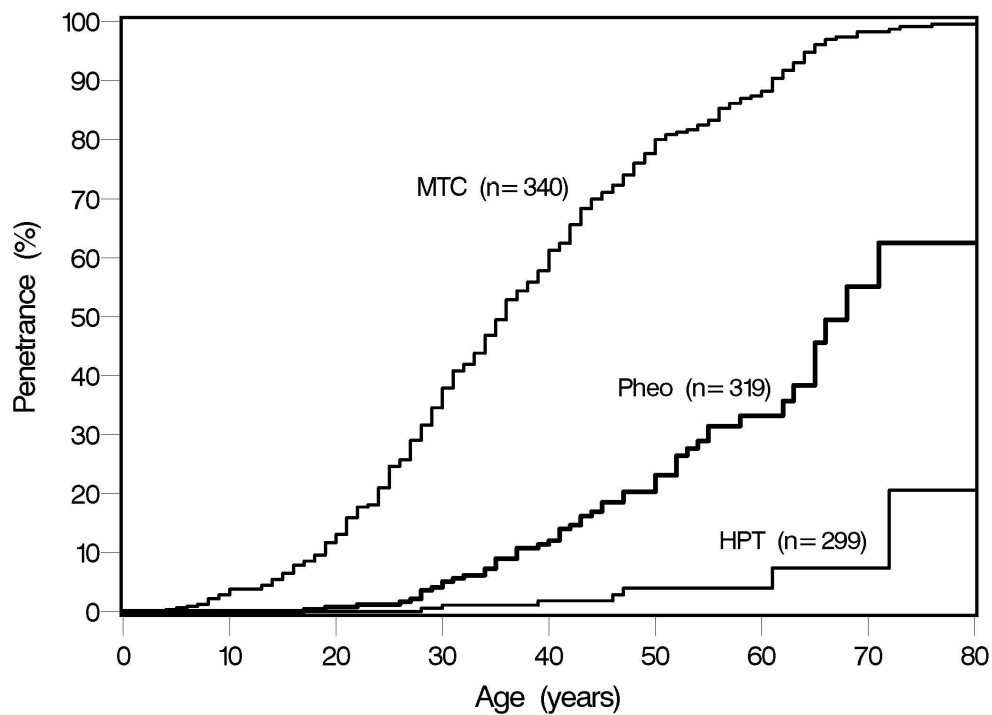
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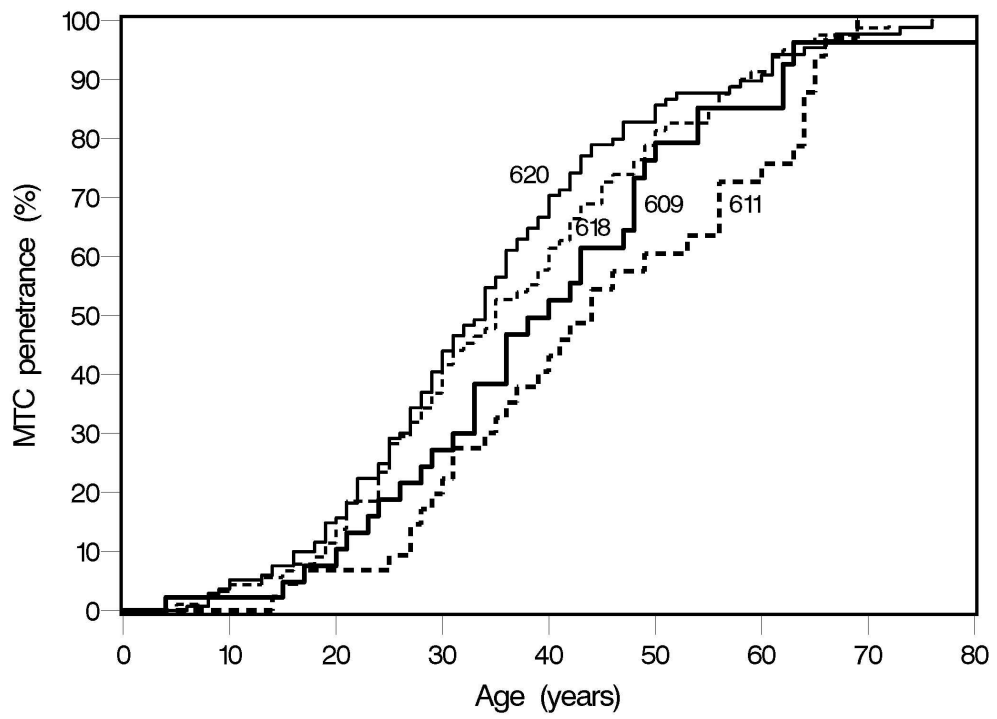


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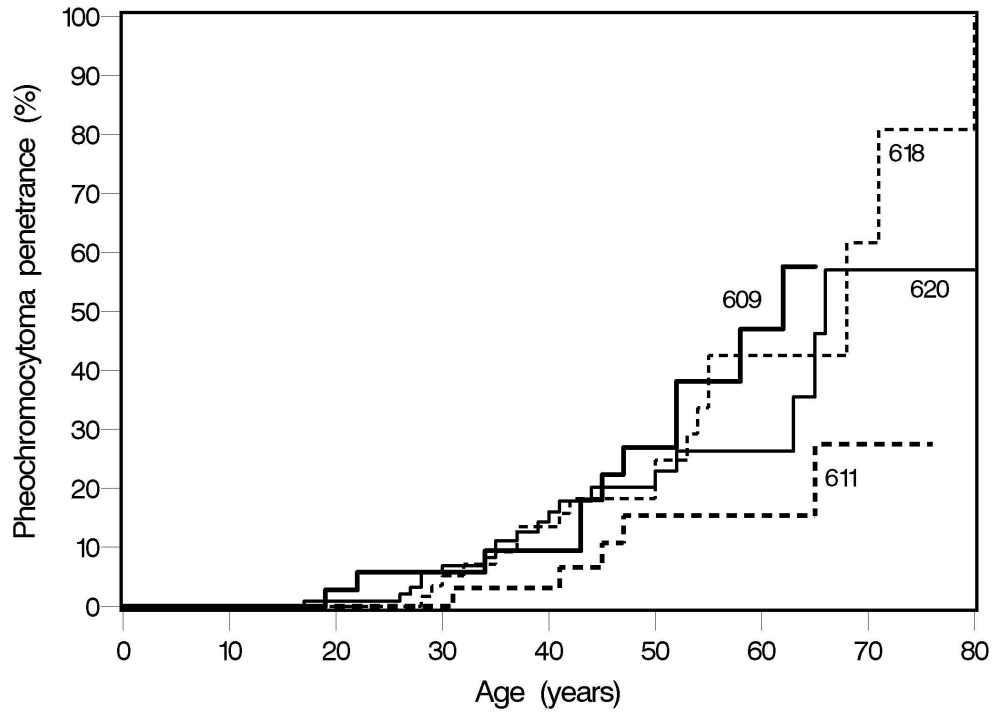
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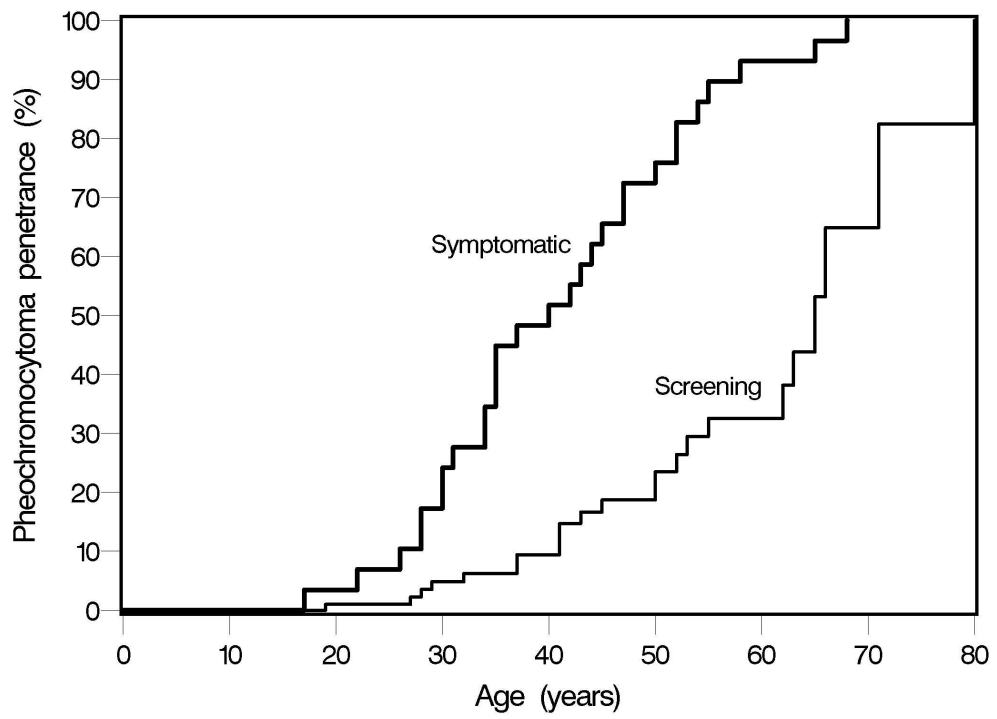


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Review

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 NIM0 n (range)	Age at M1 n (range)
609	c.1825T>C	C609R	2	7	7/0	42 (29-43)	4/7 (57)	2/7 (29)	0	0	MEN2A	1 (40)	0
	c.1825T>G	C609G	3	6	1/5	48 (4-54)	5/6 (83)	4/5 (80)	0	0	MEN2A	2 (33-48)	0
	c.1826G>A	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	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	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)
611	c.1832G>A	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	C611W	2	5	4/1	37 (14-66)	5/5 (100)	1/5 (20)	0	0	MEN2A	1 (49)	1 (37)
	c.1832G>T	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	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)
618	c.1852T>A	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	C618R	17	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	C618G	3	6	4/2	30 (21-57)	5/6 (83)	0	0	0	FMTC	0	1 (57)
	c.1853G>C	C618S	7	14	4/10	27 (9-63)	12/14 (86)	2/13 (15)	0	0	MEN2A	4 (9-50)	0

	c.1853G>A	C618Y	2	6	4/2	33 (25-55)	6/6 (100)	1/3 (33)	0	0	MEN2A	0	0
	c.1853G>T	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	C620R	24	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	C620G	1	1	0/1	22	7/13 (54)	1/1 (100)	0	4/12 (33)	MEN2A	0	0
	c.1859G>A	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	C620S	4	20	9/11	40 (14-73)	14/20 (70)	3/20 (15)	0	0	MEN2A,HSCR	5 (28-73)	0
	c.1860C>G	C620W	1	2	2/0	40 (40)	1/2 (50)	0	0	0	FMTC	0	0
	c.1859G>T	C620F	4	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		104	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 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)

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 T1-4N0or1M1, and for pheochromocytoma (pheo) and hyperparathyroidism (HPT)

Table 3 (part 1)

CCH

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

MTC 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

Table 3 continued (1)

MTC 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

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

Table 3 continued (2)

PHEO

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

HPT

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 2 Comparison of MTC-tumor-stage and outcome in the 4 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

^aTest of general association among codon groups ^bTest of trend across codons,

^c number of observations are different due to missing values