Age-related neoplastic risk profiles and penetrance estimations in multiple endocrine neoplasia type 2A caused by germ line \textit{RET} Cys634Trp (TGC $>$ TGG) mutation

Ioana N Milos$^{1,2}$, Karin Frank-Raue$^3$, Nelson Wohllk$^4$, Ana Luiza Maia$^5$, Eduardo Pusiol$^6$, Attila Patocs$^7$, Mercedes Robledo$^8$, Josefina Biarnes$^9$, Marta Barontini$^{10}$, Thera P Links$^{11}$, Jan Willem de Groot$^{11}$, Sarka Dvorakova$^{12}$, Mariola Peczkowska$^{13}$, Lisa A Rybicki$^{14}$, Maren Sullivan$^1$, Friedhelm Raue$^3$, Ioana Zosin$^2$, Charis Eng$^{15}$ and Hartmut P H Neumann$^1$

1Section of Preventive Medicine, Department of Nephrology, University of Freiburg Medical Centre, Freiburg im Breisgau, Germany
2Department of Endocrinology of the University of Medicine and Pharmacy ‘Victor Babes’, Timisoara, Romania
3Private Praxis for Endocrinology, Brückenstr. 21, Heidelberg, Germany
4Endocrine Section, Hospital del Salvador, Universidad de Chile, Santiago de Chile, Chile
5Endocrine Division, Hospital de Clinicas de Porto Alegre, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil
6Institutos de Histología, Embriología y Patología Tiroidea, Universidad Nacional de Cuyo, Mendoza, Argentina
7Molecular Medicine Research Group, Hungarian Academy of Sciences and 2nd Department of Medicine, Semmelweis University, Budapest, Hungary
8Hereditary Endocrine Cancer Group, Spanish National Cancer Center, Madrid and ISCIII Center for Biomedical Research on Rare Diseases (CIBERER), Madrid, Spain
9Endocrinology Unit, Hospital Dr Josep Trueta de Girona, Girona, Spain
10Department of Endocrinology, University Medical Center Groningen, Groningen, The Netherlands
11Department of Molecular Endocrinology of the Institute of Endocrinology, Prague, Czech Republic
12Department of Hypertension, Institute of Cardiology, Warsaw, Poland
13Department of Quantitative Health Sciences and 15Genomic Medicine Institute, Cleveland Clinic, Cleveland, Ohio, USA

(Correspondence should be addressed to H P H Neumann who is now at Medizinische Universitätsklinik, Abteilung Innere Medizin 4, Sektion für Praventive Medizin, Hugstetter Street 55, D 79106 Freiburg, Germany; Email: hartmut.neumann@uniklinik-freiburg.de)

Abstract

\textit{RET} testing in multiple endocrine neoplasia type 2 for molecular diagnosis is the paradigm for the practice of clinical cancer genetics. However, precise data for distinct mutation-based risk profiles are not available. Here, we survey the clinical profile for one specific genotype as a model, TGC to TGG in codon 634 (C634W). By international efforts, we ascertained all available carriers of the \textit{RET} C634W mutation. Age at diagnosis, penetrance, and clinical complications were analyzed for medullary thyroid carcinoma (MTC), pheochromocytoma, and hyperparathyroidism (HPT), as well as overall survival. Our series comprises 92 carriers from 20 unrelated families worldwide. Sixty-eight subjects had MTC diagnosed at age 3–72 years (mean 29). Lymph node metastases were observed in 16 subjects aged 20–72 and distant metastases in 4 subjects aged 28–69. Forty-one subjects had pheochromocytoma detected at age 18–67 (mean 36). Amongst the 28 subjects with MTC and pheochromocytoma, six developed pheochromocytoma before MTC. Six subjects had HPT diagnosed at age 26–52 (mean 39). Eighteen subjects died; of the 16 with known causes of death, 8 died of pheochromocytoma and 4 of MTC. Penetrance for MTC is 52% by age 30 and 83% by age 50, for pheochromocytoma penetrance is 20% by age 30 and 67% by age 50, and for HPT penetrance is 3% by age 30 and 21% by age 50. These data provide, for the first time, \textit{RET} C634W-specific neoplastic risk and age-related penetrance profiles. The data may facilitate risk assessment and genetic counseling.

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Introduction

Multiple endocrine neoplasia type 2 (MEN 2) is an autosomal dominant multiglandular cancer syndrome, affecting tissues of neural crest origin. It is caused by germ line mutations of the RET proto-oncogene, encoding a transmembrane tyrosine kinase receptor expressed by cells of neural crest origin, the C-cells of the thyroid, adrenal medullary cells, and enteric autonomic ganglion cells (Mulligan et al. 1993, 1994, Eng et al. 1996, Zbuk & Eng 2007). When RET mutations were found in the large majority of MEN 2 patients and a RET genotype–clinical phenotype found, this was the paradigm for the practice of clinical cancer genetics. Accurate molecular diagnosis and premorbid predictive testing became possible.

Based on the different clinical manifestations, MEN 2 is historically classified into three clinical subtypes of MEN 2A, MEN 2B, and familial medullary thyroid carcinoma (FMTC). The components of MEN 2A are MTC, pheochromocytoma, and parathyroid adenoma and/or hyperplasia (hyperparathyroidism, 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 (Farndon et al. 1986). 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 the age (Skinner et al. 1996).

The spectrum of germ line RET mutations in MEN 2 are encompassed by 58 distinct mutations that lie in 19 codons belonging to 7 exons (Peczkwksa & Januszewicz 2005; OMIM 164761). It is clear that the MEN 2B-defining mutations, M918T and A883F, portend an aggressive course, with management appropriately aggressive and preemptive (Zbuk & Eng 2007). It is also obvious and relatively consistent that the C634R, the most common mutation seen in MEN 2A, predicts for the development of MTC, pheochromocytoma, and HPT, at relatively young ages, and so management is tailored accordingly (Brandi et al. 2001, Sanso et al. 2002, Machens et al. 2003, 2005, Patocs et al. 2006, Machens & Drale 2007). However, apart from vague generalizations such as non-634 mutations are not as aggressive, 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 genotype. Take for example, the most frequently affected codon, 634, in MEN 2: studies have shown that mutations at codon 634 make the receptor tyrosine kinase constitutively active by the tendency of mutant RET monomers to undergo dimerization, a situation that may copy ligand binding. Whereas cysteine residues in wild-type RET form intramolecular disulfide bonds, the mutation of a cysteine residue leave an unpaired residue. Unpaired cysteines from two mutant RET monomers can dimerize by formation of a disulfide bond (Takahashi et al. 1998, Eng 1999). Taking into account the clinical presentation, we know that C634R has a high penetrance and is associated with the more severe forms of MEN 2A (Eng et al. 1996, Punales et al. 2003). Yet, C634Y is associated with FMTC (Eng et al. 1996, Zbuk & Eng 2007). Similarly, while investigating pheochromocytoma presentations in our Registry, we noted such a presentation with a C634W mutation, overturning the clinical rubric that MTC always occurs before other component neoplasias in MEN 2. Therefore, we believe that genotype-specific risk profiles comprising risk of developing each component neoplasia, the ages at onset, penetrance, and clinical course might be useful for tailoring more accurate clinical care. We sought to utilize C634W as a model mutation and systematically surveyed clinical information that would result in a C634W-specific risk profile.

Methods

Patients

The ascertainment of carriers with the RET C634W mutation was performed in four steps. First, C634W carriers were identified from existing registrants in the European–American Pheochromocytoma Registry, which has been described previously (Neumann et al. 2002, 2004, 2007, Schiavi et al. 2005). Second, all clinicians and researchers who had contributed to this registry were contacted for further information and updates to their C634W carriers. Third, we performed an extensive literature search with PubMed using the MeSH terms ‘C634W, Cys634Trp, RET proto-oncogene mutation, MEN 2, and genotype–phenotype correlation’. From each existing report in the published literature (Gardner et al. 1994, Lips et al. 1994, Schuffenecker et al. 1994, 1998, Biarnes et al. 1996, Frank-Raue et al. 1996, Landsvater et al. 1996, Shirahama et al. 1998, Wohllk et al. 2000, 2001, Lemos et al. 2002, Punales et al. 2003, Chung et al. 2004, Jindrichova et al. 2004, Schiavi et al. 2005, Zhou et al. 2007), we extracted the clinical data of the reported carriers of this mutation. Subsequently, we contacted the authors of these reports and requested follow-up
Fourth and finally, all known centers with clinical activities on MEN 2 or one of the component neoplasias (e.g., C634W carriers’ clinical information) to this study. We excluded double counting by contacting all authors of the utilized publications (Lips et al. 1994, Biarnes et al. 1996, Frank-Raue et al. 1996, Wohllk et al. 2000, 2001, Punales et al. 2003, Jindrichova et al. 2004, Schiavi et al. 2005). This procedure has been approved by the respective institutional review boards for human subjects’ protection in accordance with the ethical standards of each country.

Clinical data

The main inclusion criterion was a proven carrier status of the RET C634W mutation. In addition, relatives of proven mutation carriers without a genetic diagnosis were included whether a MTC or a pheochromocytoma was confirmed histologically. Furthermore, we included relatives who are obligate carriers; for example, if the index case was a mutation carrier, and her mother’s brother also has a mutation, then the mother is an obligate carrier.

Recording of demographic and clinical information from all mutation carriers was performed using a uniform format. All data were updated as of 2007 or extracted from literature reports, when direct contacts were uneventful or not possible. Approximately, 30% of the patients fell into the latter category. Demographic data included year of birth, gender, and country of residence. Family history was evaluated regarding all of the MEN 2 component neoplasias and clinical features and a pedigree constructed. Clinical data were recorded for thyroid, adrenal and parathyroid glands, and additional features of MEN 2. For all neoplasia diagnoses, we noted the age at diagnosis of the patient and whether the patient was symptomatic or asymptomatic at presentation.

For thyroid C-cell hyperplasia (CCH) and/or MTC status, we recorded preoperative calcitonin, final histopathology regarding presence of CCH and/or MTC, the number of tumors and presence or absence of lymph node metastases at initial thyroid surgery. Postoperative data included presence or absence of distant metastases. Complete remission was defined by lack of evidence of residual tumor or metastasis as revealed by imaging scans and normalization of serum calcitonin levels.

For adrenal and/or extra-adrenal paranganglial tumors, we recorded the presence or absence of symptoms and signs at presentation or anytime in the past, such as palpitation, sweating attacks, headache, and hypertension. Diagnostic data included imaging by computerized tomography (CT), magnetic resonance imaging (MRI), and metaiodobenzylguanidine scintigraphy (MIBG), and biochemical data for catecholamines or metanephrines in the 24 h urine or plasma. Histological features abstracted included the number and size of pheochromocytoma or adrenal medullary hyperplasia.

For parathyroid disease, we evaluated serum intact parathormone and presence of hyperplasia or adenoma on histology.

Statistical analyses

We estimated age-dependent penetrance for MTC, pheochromocytoma, and HPT using the Kaplan–Meier method. We used symptoms, biochemical data, imaging results, and surgical pathology in order to characterize the subjects affected or unaffected. In this aspect some subjects had incomplete data and had to be deleted. Thus, the results for MTC are based on 78 subjects, pheochromocytoma is based on 88 subjects, and HPT are based on 71 subjects.

Results

General clinical information

A total of 92 individuals, belonging to 20 families, were identified as carrying germ line RET C634W mutations. Of these carriers 52 are females and 40 males. Mean age at diagnosis of any MEN 2 component was 28 (range 3–67) years. The families and carriers originated from: Germany (25 carriers from 2 families), Spain (24 carriers from 5 families), Chile (12 carriers from 2 families), Romania (7 carriers from 2 families), Argentina (7 carriers from 1 family), Brazil (7 carriers from 1 family), Hungary (4 carriers from 3 families), The Netherlands (4 carriers from 2 families), Czech Republic (1 carrier from 1 family), and Poland (1 carrier from 1 family).

MTC

Among the total of 92 mutation carriers, thyroid operations were performed on 81 (88%). Of these patients who underwent thyroidectomy, MTC was present in 68 (84%), CCH was present in seven (9%). MTC and CCH were excluded in six patients (7%). Age at diagnosis was 3–72, mean 29 years. Asymptomatic MTC, occurring in 49 out of the 68 (72%), was diagnosed by biochemical screening or molecular genetic identification as mutation carriers during work-up of MEN 2 families or patients initially presenting with pheochromocytomas. Among the 47
asymptomatic presentations of MTC, 41% were found to have MEN 2 by genetic testing and family history. From a total of 47 patients with asymptomatic thyroid disease (MTC and CCH), who were thyroidectomized on the results of genetic diagnosis, 23 (49%) were under 20 years of age. Mean age at diagnosis of asymptomatic MTC was 24 (range 3–67) years. By contrast, symptomatic MTC was diagnosed at mean age of 36 (16–72), and CCH was diagnosed at a mean age of 11 years (range 5–17).

Age-related penetrance for MTC was 52% by age 30 and 83% by age 50 (Fig. 1).

Histological details, available from 49 patients with MTC, showed multifocal tumors in 46 (94%) and solitary in 3 (6%). Regional lymph node status was given in 61 subjects of whom 16 (26%) had lymph nodes metastases; the age of these subjects was 20–72 years. Distant metastases were present in 4 out of the 61 cases; the age of these subjects was 28–69 years.

Postoperative serum calcitonin data were available from 34 MTC patients. Of these, 25 (73%) had normal calcitonin and were regarded as free of disease. In six (24%) cases, elevated serum calcitonin indicated residual tumor tissue.

**Pheochromocytoma**

Pheochromocytoma was present in 41 out of the 92 carriers (45%). Forty-seven cases had no evidence for pheochromocytoma as shown by CT, MRI, or plasma and/or urine catecholamines. In four carriers, adrenal status was not known. For the 41 cases with pheochromocytoma, mean age at diagnosis was 36 years (range 18–67).

Age-dependent penetrance for pheochromocytoma was 20% by age 30 and 67% by age 50 (Fig. 1).

Thirty-six patients presented with symptoms, while five (aged 26, 28, 31, 43, and 67) had asymptomatic presentations. Four out of the 41 patients (10%) had more than one operation for this tumor, with inter-operation intervals ranging from 4 to 31 years.

Of the 28 patients who presented with both, MTC and pheochromocytoma, MTC was diagnosed before the pheochromocytoma in 12 subjects (21%). The interval between the diagnoses ranged from 1 to 14 years. Pheochromocytoma was diagnosed before MTC in six (21%) of the patients with intervals between the two diagnoses ranging from 1 to 10 years. In ten patients (36%) MTC and pheochromocytoma were diagnosed simultaneously. Malignant pheochromocytoma was not observed in any of the 41 patients.

**HPT**

Parathyroid data were available for 64 carriers. HPT was present in six (9%) patients. Mean age at diagnosis was 39 years (range 26–52). Penetrance for HPT was 3% by age 30 and 21% by age 50 (Fig. 1).

**Other lesions**

Cutaneous lichen amyloidosis was noted in 4 out of the 92 patients (4%).

**Survival and causes of death**

Among the 92 patients, 74 (80%) remain alive. Those who were alive at last follow-up (mean 12 years of follow-up, range 1–29) have a mean age of 35 (range 12–71). Of these 74, 60 are entirely disease free, and one has MTC-related distant metastases. We were not informed on the disease status of seven patients.

Thus, 18 out of the 92 (20%) patients had died. Mean age at death was 41 years (range 18–79). Of the 18 deceased individuals, the cause of death was known for 16. Pheochromocytoma was the dominant cause of death found in eight patients (50%). Mean age at death from pheochromocytoma was 42 years (range 18–67). Metastatic MTC was the cause of death in 4 out of the 16 patients (19%), at the ages of 21, 29, and 69 years. Two patients died due to myocardial infarction, one due to primary lung cancer, one due to an accident.

**Discussion**

Although the RET gene was shown to be the MEN 2 susceptibility gene 14 years ago (Mulligan et al. 1993), data on specific mutations of the RET gene are scarce. Ideally, for each specific RET mutation; there should...
be available age-related risks, morbidity, and mortality from each of the component neoplasias. Here, for the first time, data describing the risk profile for the \textit{RET} mutation C634W is provided based on an evaluation of 92 mutation carriers. Because of our anecdotal observations noted above, we originally hypothesized that C634W may have a less aggressive course (Punales \textit{et al.} 2003). After systematic study here, we were initially surprised that penetrance was high, i.e., \sim 98 and \sim 92\% respectively, by the age of 70 for MTC and pheochromocytoma respectively. These figures are relatively typical of classic codon 634-related MEN 2A (Brandi \textit{et al.} 2001, Machens \textit{et al.} 2003). For HPT, penetrance was 21\% by the age of 50, which is lower than in currently available reports for mutations of \textit{RET} codon 634 (Eng \textit{et al.} 1996).

Yet, of the 92 C634W carriers, 80\% remained alive at a mean 12 years of follow-up, although only 68\% were alive without disease. Disease course also seemed to vary widely, with the youngest patient having regional nodal metastases at 20 years of age, and the youngest with distant metastases at 28 years of age.

Clinicians and genetic counselors currently counsel that MTC occurs before or concurrently with the development of pheochromocytoma in almost all cases of MEN 2, and that the overwhelming cause of death in MEN 2 is metastatic MTC. To our surprise, in this series of C634W carriers, we found that 21\% with both MTC and pheochromocytoma developed pheochromocytoma before the MTC. Sixty-four percent of all deceased patients died from pheochromocytoma related complications compared with only 21\% dying of metastatic MTC. The pheochromocytoma-related mortality in our multicenter study including different countries is significant as a recent single institutional cohort reported no deaths from pheochromocytoma amongst 323 MEN 2A patients with a median follow-up of 9 years ($P<0.0001$; Quaile \textit{et al.} 2007). However, most of our patients died, before modern pheochromocytoma biochemical testing and imaging have been introduced and would have been cured in time today.

Our data are derived from mutation positive individuals from 20 families living in ten different countries in Europe or America, with 1–14 affected family members/family. One strategy to explore clinical phenotype given a genotype is to utilize all available individuals whether they are related or not. This is particularly valid when sample sizes are not particularly large, as is the case here. An important caveat is that our approach of using more than one mutation positive member/family may result in bias due to phenotypic effects from as yet unidentified shared modifier loci within any single family. If the sample size could be larger, then there are formal statistical techniques to take this into account.

Current MEN 2 practice guidelines, summarized as experts’ consensus, or lack thereof, recommend thyroidectomy at the age five for MEN 2A and considerably earlier in MEN 2B (Brandi \textit{et al.} 2001, Machens & Dralle 2007). However, recommendations based on worst-case scenarios are debatable and probably inadequate. This recommendation has been shown to be effective (Machens \textit{et al.} 2005), but it still leaves us practicing using the law of averages, with the only variation being that it is now gene based. This is not ideal as it means over treating a subset and opening them to needless complications from potentially unnecessary procedures. Recently, the need for codon-specific guidelines emerged from genotype–phenotype correlation in the context of clinical follow-up and evidence showing codon-dependent aggressiveness of \textit{RET} mutations (Machens \textit{et al.} 2003, 2005, Szinnai \textit{et al.} 2003). Furthermore, family members of patients with MEN 2 and known \textit{RET} mutation are advised to undergo genetic testing and obtain not only the result of being a carrier or not, but also the exact mutation. However, thus far, the mutation carriers and their caregivers really do not have data on which to practice in a mutation-specific manner. Here, we have begun to provide C634W-specific neoplastic risk profiles, where we found that penetrance is high for MTC and pheochromocytoma, although the clinical course can be prolonged. Importantly, in C634W carriers, pheochromocytoma occurs not infrequently before MTC and can be a significant cause of mortality.

\textbf{Declaration of interest}

We declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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