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Geographical variation in the profile of *RET* variants in patients with medullary thyroid cancer: a comprehensive review

Rui M B Maciel¹ and Ana Luiza Maia²

¹Laboratory of Molecular and Translational Endocrinology, Escola Paulista de Medicina, Universidade Federal de São Paulo, São Paulo, São Paulo, Brazil and ²Thyroid Unit, Hospital de Clínicas de Porto Alegre, Faculdade de Medicina, Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil

Correspondence
should be addressed
to A L Maia

Email
almaia@ufrgs.br

Abstract

Genetic variability in humans is influenced by many factors, such as natural selection, mutations, genetic drift, and migrations. Molecular epidemiology evaluates the contribution of genetic risk factors in the etiology, diagnosis, and prevention of a particular disease. Few areas of medicine have been so clearly affected by genetic diagnosis and management as multiple neoplasia type 2 (MEN2), in which activating pathogenic variants in the *RET* gene results in the development of medullary thyroid carcinoma (MTC), pheochromocytoma, and hyperparathyroidism in nearly 98, 50, and 25% of gene carriers, respectively. Here, we aimed to collect *RET* genotyping data worldwide to analyze the distribution and frequency of *RET* variants from a global perspective. We show that the mutational spectrum of *RET* is observed worldwide. The codon 634 variants seem to be the most prevalent, but there are differences in the type of amino acid exchanges among countries and in the frequencies of the other *RET* codon variants. Most interestingly, studies using haplotype analysis or pedigree linkage have demonstrated that some pathogenic *RET* variants have been transmitted to offspring for centuries, explaining some local prevalence due to a founder effect. Unfortunately, after almost three decades after the causative role of the germline *RET* variants has been reported in hereditary MTC, comprehensive genotyping data remain limited to a few countries. The heterogeneity of *RET* variants justifies the need for a global effort to describe epidemiological data of families with MEN2 to further understand the genetic background and environmental circumstances that affect disease presentation.

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Invited Author's profile

Dr Ana Luiza Maia is Full Professor of Medicine at the Endocrine Division, Medical School, Universidade Federal do Rio Grande do Sul, and Head of the Thyroid Unit, Hospital de Clínicas de Porto Alegre, Brazil. Dr Maia leads studies of molecular diagnosis and genotype–phenotype correlations in Multiple Endocrine Neoplasia Type 2 patients in Brazil. Her research interests also includes thyroid hormone metabolism and clinical and pathological features of patients with thyroid carcinomas.

Introduction

RET pathogenic variants (1) cause multiple endocrine neoplasia type 2 (MEN2), an autosomal-dominant hereditary syndrome characterized by an association with endocrine tumors (2, 3, 4, 5, 6, 7, 8). MEN2 is classified into subtypes, MEN type 2A and MEN type 2B according to its clinical manifestations, which are distinguished by a high penetrance of medullary thyroid carcinoma (MTC) and, to a lesser extent, pheochromocytoma (PHEO) and primary hyperparathyroidism (PHPT) (8, 9, 10).

The *RET* gene encodes a transmembrane receptor, and gain-of-function missense variants in the extracellular or cytoplasmic regions of the *RET* protein promote continuous phosphorylation of a distinct set of tyrosine residues, triggering intracellular signaling pathways for cell survival, differentiation, and proliferation (8, 9, 10). The several reported *RET* pathogenic variants and their corresponding locations are illustrated in Fig. 1. The oncogenic codon-specific *RET* pathogenic variants present

strong correlations with the MEN2 phenotype (7, 8, 9, 10, 11). A large proportion of MEN2A patients presenting with PHEO or PHPT, or both, have *RET* pathogenic variants in the cysteine-rich extracellular domain, particularly in exons 10 and 11 (8, 9). Interestingly, it seems that the disease phenotype and aggressiveness might vary accordingly with specific codon 634 amino acid exchanges (12, 13). *RET* variants in codons 609, 611, 618, and 620 are also frequently occurring genetic alterations. Amino acid changes in the intracellular domain of *RET* in exon 13 (codons 768, 790, and 791), exon 14 (codons 804 and 844), and exon 15 (codon 891) are usually associated with an isolated MTC phenotype. The rare association with cutaneous amyloidosis occurs almost exclusively in carriers of codon 634 variants (14), while Hirschsprung's disease has been observed only in kindreds with variants in codons 609, 611, 618, and 620 (15). No *RET* variants are found in 2–5% of apparently hereditary MTC cases (16, 17). Of note, whole-exome sequencing has recently identified a germline *RET* variant in two siblings with

Figure 1

(A) Left: *RET* protein and domains; middle: *RET* variants and their corresponding locations (in bold, variants unambiguously pathogenic); (B) *RET* pathogenic variants and their corresponding codons and relative incidence of MEN2-associated neoplasias. Pheo, pheochromocytoma; HPH, hyperparathyroidism.

hereditary *RET*-negative MTC (18). The vast majority of patients with MEN2B harbor the *RET* variant p.Met918Thr, and a recent international multicentric study in which 345 individuals were evaluated showed the importance of increasing awareness of the extraendocrine symptoms characteristic of this syndrome (19).

MTC occurs in more than 95% of MEN2 patients, and several studies have shown that early thyroidectomy is an effective treatment as it prevents or cures MTC before it spreads (7, 8, 9, 10, 11). In addition, a correlation between the affected *RET* codon and disease aggressiveness and progression has been demonstrated (7, 8, 9, 10, 11, 20, 21). Therefore, guidelines issued by several societies, as the American Thyroid Association (ATA), European Thyroid Association, and Brazilian Society of Endocrinology and Metabolism stratify *RET* pathogenic variants into three levels, highest, high, and moderate risk, based on the risk and timing of the development of MTC (21, 22, 23, 24, 25).

Although customarily deemed a rare disease, the earlier diagnosis with the consequent finding of asymptomatic carriers of MEN2 has increased its prevalence, from 1 patient per 200 000 or 500 000 inhabitants to nearly 1:80 000 inhabitants (26). As an example, in Norway, a country where epidemiological data from the entire population have recently been published, the incidence of patients with *RET* variants associated with MEN2 was 1:66 438 live births, while the prevalence of MEN2 was 1:79 462 (27).

Geographic spectrum of *RET* variants

Genetic variability in humans is influenced by many factors, such as natural selection, mutations, genetic drift (which includes founder and bottleneck effects), and migrations. Frequent germline genetic variants tend to be older, while rarer genetic variants have probably appeared in more recent times (28). In large populations, selection tends to have a greater impact on allele frequency than on genetic drift. In contrast, genetic drift plays a more important role in small populations. Thus, many pathogenic variants must have appeared centuries ago and continue to be passed on to new generations (29).

Several studies have shown that the *RET* variant profile may vary according to geographical area (26, 27, 30, 31, 32, 33, 34, 35, 36, 37). In fact, recent ethnographic fieldwork and haplotype analysis traced founder variants from Brazil back to the Iberian Peninsula and Greece (33, 35), while Machens *et al.* attempted to unearth European ancestral heritage using German *RET* data (36). Moreover,

an international consortium found that the penetrance of PHEO in MEN2 patients carrying *RET* variants in exons 10 and 11 is lower in countries from South America than in Europe, indicating that other genetic or environmental modifiers could explain this difference (37).

Here, we aimed to collect worldwide *RET* gene genotyping data to analyze the distribution and frequency of *RET* pathogenic variants from a global perspective. Thus, we conducted a review of the literature, employing a PubMed/Medline search using the following key words: *RET* Mutation in all affected codons, *RET* Epidemiology, *RET* and multiple countries, MEN2 Epidemiology, MEN2 guidelines, and Medullary Thyroid Cancer. We also searched for *RET* in various databases (the Cancer Genetics Web, OMIM from Johns Hopkins University, International Cancer Genome Consortium, Cancer Genome Anatomy Project, COSMIC Sanger Institute, and Leiden Open Variation Database and Arup). Additionally, the references of included studies were screened for additional publications. No language or time restriction was adopted. The last search was performed on January 7, 2021.

Largest series on MEN2-related *RET* pathogenic variants from Germany, France, Italy, United Kingdom, Brazil, Japan, and United States of America

Table 1 compares the results of *RET* pathogenic variants causing MEN2 in the largest published series, originating from Germany ($n = 533$) (9, 26, 29, 36, 38, 39), France ($n = 437$) (39, 40, 41), Italy ($n = 237$) (17, 31, 32, 42), United Kingdom (UK) ($n = 110$) (43), Brazil ($n = 554$) (44), United States of America (USA) ($n = 403$) (45, 46, 47, 48, 49, 50, 51, 52), and Japan ($n = 390$) (53, 54, 55, 56, 57).

The pathogenic variants at codon 634 (p.Cys634Phe, p.Cys634Gly, p.Cys634Arg, p.Cys634Ser, p.Cys634Trp, and p.Cys634Tyr) are the most prevalent, found in between 30 and 50% of affected patients; the p.Val804Met variant is the second in frequency in France, Germany, and Italy, followed by p.Leu790Phe in Germany and France, p.Ser891Ala in Italy, and p.Gly533Cys and p.Cys620 in Brazil. The frequency of variants occurring at codon 618 is between 6 and 10% in European countries. The other pathogenic variants are found in less than 10% of MEN2 patients, mainly at codons Cys609, Cys611, and Cys630. The MEN2B phenotype, caused by the variant p.Met918Thr, was similarly distributed in all seven countries, corresponding to 4.6–13.5% of affected patients.

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Table 1 Relative frequency of MEN2-related *RET* pathogenic variants in major consolidated series.

ATA risk category	Amino acid change	Relative frequency, n (%)						
		Germany (n = 567)	France (n = 437)	Italy (n = 237)	UK (n = 110)	Brazil (n = 554)	Japan (n = 390)	USA (n = 403)
Highest	p.Met918Thr	39 (7.4)	30 (6.9)	17 (7.2)	15 (13.5)	32 (5.8)	18 (4.6)	46 (11.4)
High	p.Cys634(F/G/R/S/W/Y)	184 (34.8)	144 (32.9)	86 (36.2)	32 (29)	262 (47.0)	144 (37.0)	0
	p.T338I	0	1 (<1)	1 (<1)	0	0	1 (<1)	163 (40.4)
	p.Ala883Phe*	1 (<1)	2 (0.5)	0	2 (1.8)	0	2 (0.5)	0
Moderate	p.Cys515(F/S)	0	4 (0.9)	1 (<1)	0	0	4 (1)	0
	p.Cys531R	0	2 (0.5)	0	0	0	2 (0.5)	0
	p.Gly533Cys	1 (<1)	3 (0.6)	0	3 (0.7)	59 (10.6)	3 (0.7)	7 (1.8)
	p.Cys541Arg	0	1 (<1)	0	0	0	1 (<1)	0
	p.Cys609(F/G/R/S/W)	7 (1.3)	5 (1.1)	6 (2.5)	5 (4.5)	12 (2.2)	6 (1.5)	44 (10.9)
	p.Cys611(F/Y)	42 (7.9)	12 (2.7)	1 (<1)	0	12 (2.2)	24 (6.2)	8 (2)
	p.Cys618(F/G/R/G/S/Y)	53 (10.0)	29 (6.6)	15 (6.3)	12 (11)	11 (2.0)	68 (17.4)	43 (10.7)
	p.Cys620(F/R/S/Y)	38 (7.2)	31 (7.1)	9 (3.8)	11 (10)	56 (10.1)	39 (10)	38 (9.4)
	p.Cys630Arg	4 (0.7)	1 (<1)	4 (1.7)	0	2 (<1)	11 (2.8)	2 (<1)
	p.K666(E/N/T)	0	6 (1.4)	1 (<1)	1 (<1)	0	1 (<1)	11 (3.2)
	p.Glu768Asp	10 (1.9)	10 (2.3)	9 (3.8)	3 (2.7)	14 (2.5)	19 (4.9)	0
	p.Leu790Phe	61 (11.6)	43 (9.9)	8 (3.4)	4 (3.6)	4 (<1)	1 (<1)	2 (<1)
	p.Val804 (L/M)	67 (12.7)	92 (21.0)	52 (21.9)	14 (13.0)	46 (8.3)	12 (3.0)	29 (7.2)
	p.Ala883Thr	0	1 (<1)	1 (<1)	0	0	1 (<1)	0
	p.Ser891Ala	25 (4.7)	20 (4.6)	23 (9.7)	8 (7.3)	23 (4.1)	10 (2.6)	10 (2.5)
	p.Ser904Phe	1 (<1)	0	1 (<1)	4 (3.6)	0	0	0
	p.Met918Val	0	1 (<1)	2 (<1)	0	21 (3.8)	0	0

*The 2015 ATA categorization of p.Ala883Phe as carrying “very high risk” is no longer current, as per Mathiesen *et al.* 2017 (80).

French data represent the results of a multicenter observational study led by the French Group of Endocrine Tumors including data from 18 different centers (40, 41). In addition to the 437 patients depicted in Table 1, the French Group has included new patients ($n = 36$) who present variants not classified by the ATA, thus far considered probably pathogenic, benign, or a variant of unknown significance; the clinical follow-up of these patients and further phenotype-genotype information will be necessary to classify the MTC risk profile (41). In addition, the data included three different double variants (p.Val804Met; p.Arg844Leu), (p.Lys603Gln; p.Leu790Phe), and (p.Asp631Tyr; p.Tyr791Phe).

The data from Germany on *RET* genotyping are comprehensive and up-to-date (9, 26, 29, 36, 38, 39). It is interesting to note that one of the German centers, when describing their 40 years of experience with 263 MTC patients (9), presented some differences in *RET* genotypes in comparison with the total number of cases in Germany (39); thus, in this series, there were more patients with p.Cys634Tyr ($n = 49$) than patients with p.Cys634Arg ($n = 30$) and an equal number of patients with p.Leu790Phe and p.Ser891Ala variants (9, 39), further demonstrating heterogeneity in *RET* genotypes throughout Germany (36).

The Italian records on *RET* genotyping are also comprehensive and current (17, 31, 32, 42). It is

interesting to note the high prevalence of the pathogenic variant p.Val804Met in the Italian data and the founder effects of variants p.Ser891Ala in northern Italy (58) and p.Val804Met in Sardinia (59).

Since France, Italy, and Germany are neighboring countries in Western Europe and all have comprehensive DNA-based screening programs for MEN2, it is somewhat anticipated that their data would be relatively similar; however, there are few appealing differences. The p.Val804Met variant is more frequent in Italy (20.2–25.6%) and France (17.2–21.4%) than in Germany (10%) (17, 31, 32, 38, 39, 42). A high prevalence of the p.Val804Met variant was primarily reported in a Sardinian study (59% prevalence), and this finding was explained as an outcome of the role of genetic drift and the founder effect in the history of that population, who lived isolated for several centuries (59). Nevertheless, this explanation is not true for continental Italy, since the pattern of *RET* gene single-nucleotide polymorphisms in index cases harboring p.Val804Met is different (31).

Another interesting observation in these series is that the pathogenic variant p.Ser891Ala is more prevalent in Italy (9.7%) than in Germany or France (4.7 and 4.6%, respectively). This is apparently due to a founder effect in an isolated mountainous region in northern Italy (58). The authors noticed an impressive percentage of

patients harboring the p.Ser891Ala variant in their local series (54.9%) when compared with the percentage found in Italy for p.Val804Met (13.7%). The hypothesis of a common ancestor was then investigated, and a common haplotype identified, as demonstrated by accurate research on church and municipal archives and regional clustering of mutations in kindreds in two areas, the Seriana Valley, in the province of Bergamo, and the Plain of Brescia. The reconstruction of the genealogical tree confirmed that family residents in Brescia were descendants of immigrants from the Seriana Valley in the 1600s, supporting the hypothesis that the p.Ser891Ala variant was most likely introduced by a founder pathogenic variant (58). Recent studies from Italy (not included in Table 1) from two distinct centers have shed light on these observations. The first one, from Pisa, including 353 patients, identified 42 different germline missense variants with a distribution of affected codons similar to those found in previous reports; in addition, some rare variants, among them Ala883Thr, were found in nine patients (17). The second study, from Bologna and Parma, analyzed 72 MTC patients and identified a likely pathogenic variant, p.Ser904Phe, found in eight carriers in a kindred (60).

On the other hand, p.Leu790Phe is more common in Germany (11.6–13.6%) and France (9.9%) than in Italy (3.4%). Machens *et al.* addressed this issue in a fascinating study that disclosed European ancestral heritage, showing the differences among these abovementioned *RET* variants in present-day Germany (36). The authors analyzed the *RET* data from 189 German families and observed that variants p.Val804Met and p.Leu790Phe are distributed in different historical German regions: *RET* p.Val804Met families are located mainly in the so-called ‘Roman’ historic settlement area, whereas *RET* p.Leu790Phe families are more prevalent outside than inside the historic ‘Roman’ settlement area. Because the *RET* p.Leu790Phe variant was found in at least 19 families from France and 8 families from Italy, studies from these countries may be ideally suited to elucidate the European ancestry of the *RET* p.Leu790Phe variant. The authors also showed that the benign *RET* variant p.Tyr791Phe shows a significant association with the ‘Slavic’ historic settlement area in present-day Germany. Notably, some pathogenic *RET* variants have been transmitted for centuries, as demonstrated for the p.Cys634Tyr variant in the fascinating first description of PHEO in Germany in 1886 (61) and the p.Cys611Phe variant in a study from Germany in 1876 (39).

Comparing the data of France, Italy, and Germany with those from Brazil, we observe similarities as well

as differences. While variants p.Met918Thr, p.Cys620X, p.Glu768Asp, and p.Ser891Ala present similar proportion in these countries, the p.Leu790Phe variant is more frequent in Germany and France ($P < 0.01$), the p.Val804Met mutation is more frequent in Italy and France ($P < 0.05$), and p.Ser891Ala in Italy. On the other hand, pathogenic *RET* variants p.Gly533Cys and p.Met918Val are mainly found in Brazil. For codon 634 mutations, the relative frequency in Brazil (47.0%) was higher than those found in France, Germany, Italy, UK, Japan, or USA ($P < 0.05$) (Table 1). One potential explanation could be the fact that *RET* screening in Brazil is not yet comprehensive, and patients with variants at codon 634 develop the disease earlier, a scenario comparable with that of the early European data on *RET* variants causing MEN2, when codon 634 variants were found in between 63 and 67.6% of MEN2 patients (11, 62). Nevertheless, it should be noted that codon 634 mutations are found in nearly 90% of MEN2 patients in southern Brazil, where molecular diagnosis has been performed for over two decades. Another interesting observation is that p.Cys634Arg is the most frequent variant in Italy, France, and Germany, whereas in Brazil, the most frequent is p.Cys634Tyr. In the German database, among 184 patients harboring codon 634 variants, 84 (45.6%) had p.Cys634Arg and 52 (28.2%) had p.Cys634Tyr. In the French database, among 144 patients with codon 634 variants, 53 (36%) had p.Cys634Arg and 46 (32%) had p.Cys634Tyr. In the Italian database, among 86 patients with codon 634 variants, 34 (40%) had p.Cys634Arg and 28 (33%) had p.Cys634Tyr (17, 39, 41, 42). On the other hand, among 262 Brazilian patients affected by a variant at codon 634, 166 had Cys634Tyr (63.4%), whereas 74 (28.2%) had Cys634Arg. Interestingly, the Tyr791Phe variant co-occurred with the Cys634Arg (Cys634arg/Tyr791Phe) in 47 patients (44).

The findings that *RET* pathogenic variants in Brazil display similarities as well as differences from those observed in Europe might be justified by the genomic ancestry of Brazilians, presenting individual proportions of European, African, and Amerindian ancestry in their mosaic genome (63, 64, 65).

The p.Gly533Cys *RET* pathogenic variant was firstly described in the largest MEN2A family studied so far; they emigrated from Barcelona, Spain, to Brazil at the end of the 19th century (66, 67, 68). Some years after this report, Greek researchers found that p.Gly533Cys was the most prevalent *RET* variant in Greece (69, 70, 71), a fact that has been anticipated, since the Mediterranean Sea has always been a place of frequent migratory currents

(66, 73). This variant has also been described in a family with Greek ancestors living in the USA (74), in a patient from Slovenia living in Ireland (75) and, more recently, in gene carriers in France (39, 41), Germany (9), and the UK (43). It has been hypothesized that these families would have a common ancestor, as several Greek city states were scattered throughout the Mediterranean since ancient times (73). Cunha *et al.* endorsed this hypothesis by showing that Brazilian (originally from Spain) and Greek patients have a common ancestor (35). This interesting finding suggests that, historically, the most likely origin of this variant was Greece and that the Greek migratory currents have taken the variant to the Iberian Peninsula.

Another finding of the Brazilian series was the identification of the p.Met918Val variant (33). Codon 918 is typically related to MEN2B when the first mutation caused by a methionine substitution by threonine at this codon (p.Met918Thr) was described (4, 5, 6). However, the clinical presentation of MTC related to p.Met918Val and the results of *in silico* and *in vitro* analyses (76) indicate that this is a moderate-risk variant (24, 33). At first, it was supposed that these families were isolated, but the haplotype study showed that all affected patients had a common ancestor, and through historical and mathematical studies, the existence of a pioneer who emigrated from Portugal to Brazil in 1700 was confirmed, corroborating the hypothesis that this variant accompanied the population of this region for approximately 350–400 years and that these supposedly different families belong to a single family fragmented over the centuries (33). There are now reports of the p.Met918Val variant in Italy, France, and Russia (31, 41, 77).

Recently, a large study of the *RET* genotypes and MEN2-related clinical features of 1,058 index MTC patients from the UK was published (Table 1) (43). The authors found *RET* pathogenic variants in 110 patients (10.2%), the majority occurring at codon 634 ($n = 32$, being 11 p.Cys634Arg; 11 p.Cys634Thyr; 6 p.Cys634Gly; 2 p.Cys634Ser; 1 p.Cys634Phe, and 1 p.Cys634Trp). Fifteen MEN2B patients presented the pathogenic variant p.Met918Thyr. Variants p.Gly533Cys and p.Ala883Phe were also reported (43). A retrospective review of prophylactic thyroidectomy in children who underwent genetic testing confirmed the high prevalence of codon 634 variants (78).

As mentioned, differently from France, Germany, Italy, UK, Brazil, and Japan where the results of *RET* variants were consolidated by national studies, the results of *RET* variants from USA represent our compilation of

published reports (45, 46, 47, 48, 49, 50, 51, 52, 74) (Table 1). Actually, patients from USA with MEN2 have been included in the International *RET* Mutation Consortium since the first studies on *RET* genotyping (7). In our compilation, we have included the following studies: the first publications on *RET* variants in American patients reporting the outcome of thyroidectomy from MD Anderson Cancer Center (47); the series of 50 MEN2A patients with variants at codons 634 (38%), 620 (32%), 618 (22%), 609 (6%), and 611 (2%) from Duke University (48); a large series including 262 MEN2A patients from the MD Anderson Cancer Center, showing variants at codon 634 (52%), codon 609 (14%), codon 618 (12%), codon 620 (8%), p.Val804Met (6%), codon 891 (4%), and codon 611 (3%) (49). We have also included a small series of 29 patients with medullary microcarcinoma and C-cell hyperplasia from the Massachusetts General Hospital showing a higher prevalence of p.Val804Met (48%) (50) and a series of 38 children and adolescents from NIH presenting MEN 2B caused by the variant p.Met918Thr (46).

There are also interesting observations on rare variants causing MTC, such as p.Gly533Cys (74) and p.Lys666Arg (51). Another interesting paper shows 12 rare germline *RET* variants in a large series of 6700 cases from the Mayo Clinic and Quest Diagnostics; segregation within families suggests that p.Lys666Glu (MTC and PHEO) and Thr636insGlu-Leu-Cys-Arg; Thr636Pro, a 13bp insertion and 1bp deletion (MTC), respectively, are disease-causing variants (45). Finally, it is important to cite that Arup Laboratories, University of Utah, has developed and maintains a database of the 199 *RET* variants found worldwide, with genotype, codon change, protein change, classification according to pathogenicity, MEN 2 phenotype, first reference, and comments (www.arup.utah.edu/database/MEN2/MEN2_welcome.php); however, the database does not specify the number of affected patients for each variant or their geographical origin (52).

In the largest MEN2 patient series from Japan, Imai *et al.* (53) showed the distribution of *RET* variants in 390 patients with MEN2. Codon 634 was the most affected (37%), followed by codon 618 (17.4%) and 620 (10%). *RET* pathogenic variants at codons 609, 610, 611, 620, 768, 790, 804, and 891 have also been reported among Japanese (Table 1). Other studies have shown similar *RET* codon variant distribution (54, 55). Interestingly, Japan presents the lowest rate of MEN2B patients among the countries. A newly described mutation at codon 666, p.Lys666Arg, has also been recently found in Japan. Codon 666,

located in the *RET* intracellular juxta-membrane domain, is a highly conserved residue among species (56). Some missense variants at *RET* codon 666 have been reported in MTC patients: p.Lys666Glu in the USA and Italy (45, 79), p.Lys666Met in Italy (31), and p.Lys666Asn in Italy and the USA (45, 80). Interestingly, Japanese researchers have described a novel combination of tandem *RET* variant (p.Gln781Arg/p.Val804Met) in an MEN2B-like patient; *in silico* analysis showed a high prediction score, which was compatible with the patient's clinical phenotype (57).

The MEN2B phenotype, caused mainly by the variant p.Met918Thr, was equally distributed in all larger series, corresponding to 4.6 to 13.5% of affected patients (Table 1). The categorization of variant p.Ala883Phe as carrying 'very high' risk is no longer current, since an international cohort study has demonstrated that these patients, even presenting MEN2B phenotype, have a more indolent natural course when compared with that of p.Met918Thr (81).

Comprehensive *RET* database incidence and prevalence in Norway and Denmark

Norway and Denmark have a complete cancer registry database, which includes the number of MEN2 patients and their respective *RET* genotypes. In addition to providing relevant information on *RET* variants in geographic areas, these studies further illustrate that the prevalence of the distinct *RET* variants might be particular for every country, even in those that are very close geographically, linguistically, and historically, such as Norway and Denmark. Indeed, it may be worthwhile mentioning that the kingdoms of Norway, Sweden, and Denmark once were united under one single Danish monarch (Kalmar Union, from 1397 to 1523, until the Kingdom of Sweden broke away).

Norway has a population of 5.1 million inhabitants and 237 patients with MTC (4.2% of all thyroid cancer patients), of whom 67 have MEN2A. The incidence of MTC grew from 0.18 to 0.25 for every 100 000 inhabitants/year between 1994 and 2016, or 1/66 438 live births per year (27). The prevalence of MEN2A is estimated to be between 1:73 786 and 1:79 462 (27, 82). The affected *RET* codons in Norwegian MEN2A patients are, in order of frequency, 634 (50%), 611, 804, 781, 790, 618, and 620 (27, 82).

Interestingly, Denmark, shows differences in the incidence, prevalence, and distribution of MTC-related *RET* variants when compared with Norway. Hence,

Denmark, for a population of 5.7 million inhabitants, has approximately 476 patients with MTC, 25% of whom have hereditary MTC (34, 83, 84, 85, 86, 87). The incidence is 28 per million live births per year, and the prevalence is 24 per million. The most prevalent *RET* variant is p.Cys611Tyr (68%; founder effect, see below), followed by p.Cys618Tyr (6%), p.Cys611Trp (5%), p.Cys620Arg (4%), p.Asp631Tyr (4%), p.Cys634Arg (2%), p.Val804Met (2%), p.Leu790Phe (2%), p.Cys618Phe (1%), and p.Cys634Tyr (1%) (34, 83, 84, 85, 86).

Regarding the founder effect of *RET* variant p.Cys611Tyr in Denmark, Mathiesen *et al.* studied 12 apparently unrelated families and demonstrated that all shared a common haplotype, suggesting a recent common ancestor (86). Carriers of the p.Cys611Tyr variant have also been reported in Germany, the Netherlands, Portugal, the Czech Republic, Hungary, Brazil, China, Japan, Iran, and the USA. Although unclear, one might speculate that some of these carriers, especially those from countries in proximity to Denmark, might be related to Danish families. Of interest, recently, in Denmark the first case of a European family harboring a *RET* variant (p.Cys620Arg) with children born after a preimplantation genetic test for monogenic disorders (85) was reported.

A complete database of MEN2B patients is also available in Denmark. In the period from 1941 to 2014, 12 patients were included, 7 of whom harbored the Met918Thr mutation and 1 harbored the Ala883Phe mutation (4 patients died before *RET* testing was available) (81, 87). The prevalence in 2015 was 1.06 patients/million; the incidence from 1970 to 2000 was 2.6 per million live births per year.

Summary of other published *RET* genotype data from countries across the 5 continents

Table 2 summarizes published data of *RET* pathogenic variants from countries across the five continents. The majority of studies includes a relatively small number of patients and analyzes only exons 10, 11, and 13–16. Nevertheless, some interesting observations about founder effects, uncommon *RET* variants, or disease phenotype described in some countries deserve to be highlighted.

In Cyprus, the p.Cys618Arg is by far the most prevalent pathogenic variant due to a possible founder effect (88). There is also a fascinating description of a Swedish family harboring the p.Cys618Arg mutation, which was mapped over six generations with differences in tumor aggressiveness (89).

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Table 2 *RET* pathogenic variants from countries across five continents (excluding Brazil, Denmark, France, Germany, Italy, Japan, Norway, UK, and USA).

Continent	Country	<i>RET</i> pathogenic variants reported	References
Africa	Morocco	p.Cys634Arg/Phe/Trp	107, 108
	South Africa	p.Cys634Ser/Phe/Arg, p.Cys620Trp/Arg	109, 110
Americas	Argentina	p.Cys634Arg/Thr/Phe (79%), p.Met918Thr, p.Cys611Thr	111, 112
	Canada	p.Met918Thr	113
	Chile	p.Cys634Arg/Trp (55%), p.Cys618Arg, p.Cys620Arg, p.Val804Met, p.Ser891Ala, p.Met918Thr	114
	Mexico	p.Cys634Phe, p.Met918Thr	115
Asia	China	p.Val292Met, p.Ser409Tyr, p.Tyr606Cys, p.Cys618Tyr/Arg/Ser, p.Cys620Tyr, p.Cys634Tyr (75%), p.Asp707Glu, p.Leu790Phe, p.Ser891Ala, p.Met918Thr	96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106
	India	p.Cys609Arg, p.Cys611Tyr, p.Cys618Gly, p.Asp631Tyr, p.Cys634Arg/Tyr/Gly/Ser/Thr (most prevalent), p.Val804Met, p.Ser891Ala, p.Leu790Phe, p.Met918Thr	116, 117, 118, 119, 120
	Iran	p.Cys618Tyr/Arg/Phe/Ser, p.Cys620Arg/Phe, p.Cys630, p.Cys634Arg/Tyr, p.Val804Met, p.Met918Thr	121, 122, 123, 124
	Israel	p.Cys618Arg, p.Cys634Arg, p.Val804Met, p.Met918Thr	125, 126
Europe	South Korea	p.Cys618Arg, p.Cys634Tyr/Arg (37.1%), p.Met918Thr, p.Cys 611*, p.Cys618*, p.Cys620*, p.Glu768Asp, p.Leu790Phe, p.Val804Met	127, 128
	Saudi Arabia	p.Cys 611*, p.Cys618*(46.6%), p.Cys634*, p.Cys620Ser, p.Met918Thr	129, 130
	Austria	p.Cys611Tyr/Trp, p.Cys618Ser, p.Cys620Tyr, p.Cys634Tyr/Arg/Ser/Trp, p.Leu790Phe, p.Val804Leu, p.Ser891Ala	131, 132
	Cyprus	p.Cys618Arg (69.2%), p.Cys634Phe, p.Met918Thr	88
	Czech Republic	p.Cys634Arg/Ser/Trp/Tyr (>50% cases), p.Cys609Tyr, p.Cys611Tyr, p.Glu768Asp, p.Tyr791Phe, p.Val804Met, p.Met918Thr	133
	Greece	p.Gly533Cys (36.3%), p.Cys618Arg/Tyr/Ser (32.8%), p.Cys620Tyr/Arg/Phe, p.Cys634Arg/Tyr/Phe, p.Leu804Met, p.Met18Thr	35, 69, 70, 71, 72, 134
	Ireland	p.Gly533Cys, p.Cys634Arg	75, 135
	Northern Ireland	p.Met18Thr	136
	Poland	p.Cys609Phe/Arg/Tyr, p.Cys618/Gly/Phe, p.Cys620Arg, p.Cys634Arg p.Glu768asp, p.Val804Leu, p.Met918Thr	137, 138, 139
	Portugal	p.Cys515Trp, p.Cys531Arg, p.Cys609Arg, p.Cys611Tyr, p.Cys620Arg, p.Cys634Arg/Tyr, p.Thr636Met, p.Val804Leu, p.Met918Thr	140, 141, 142, 143, 144
	Russia	p.Cys634Arg, p.Ser891Ala, p.Ala639Gly, p.Ala641Arg, p.Arg886Gln, p.Ser922Phe, p.Met918Val	77, 145, 146, 147, 148
	Serbia	p.Cys634Phe/Trp/Tyr/Arg (66.7%), p.Val804Met, p.Cys618Arg, p.Leu790Phe	149
	Slovenia	p.Cys634Tyr, p.Cys634Arg, p.Cys634Gly, p.Cys618Arg, p.Cys618Ser, p.Leu790Phe, p.Ala639Thr	150, 151
	Spain	p.Cys609Ser, p.Cys611Phe, p.Cys618Arg, p.Cys620Ser, p.Ser589Cys, p.Cys634Tyr/Arg, p.Glu768Asp, p.Val804Met, p.Ser891Ala, p.Met918Thr	90, 91, 92, 93, 94, 95
Oceania	Sweden	p.Cys618Arg	89, 152
	Switzerland	p.Cys618Ser, p.Cys630Phe, p.Cys634Tyr/Arg/Ser/Phe, p.Met918Thr	62, 153, 154
	Turkey	p.Cys634Arg (54.9%), p.Val804Met (25.4%)	155, 156
	Australia	p.533, p.Cys618*, p.Cys620*, p.Cys634*, p.Val804Met, p.Met918Thr	157, 158

*Amino acid exchanged not specified.

In the last 25 years, there have been several isolated publications on *RET* pathogenic variants in Spain but not a comprehensive review. It seems to be a preponderance of mutations at codon 634, with the majority of families harboring the p.Cys634Tyr variant instead of p.Cys634Arg (90, 91, 92, 93, 94), which is different from the rest of Europe. Interestingly, a study comparing both variants demonstrated that p.Cys634Arg is more aggressive than p.Cys634Thy (93), a finding previously reported

in a Brazilian cohort (12). The rare pathogenic variant p.Ser589Cys has been reported in a Spanish family, now categorized as a moderate risk of the disease (95).

Interestingly, the first paper on *RET* genotyping from China indicated that pathogenic variants are restricted to those occurring at codon 634, causing MEN 2A syndrome in 75% of patients (with a preponderance of p.Cys634Tyr), and p.Met918Thr, resulting in MEN2B syndrome (96). Nevertheless, the widespread use of molecular

diagnosis reported other variants, such as p.Tyr606Cys, p.Cys618Tyr, p.Cys618Arg, p.Cys618Ser, p.Cys620Tyr, p.Leu790Phe, and p.Ser891Ala (97, 98, 99, 100, 101, 102). Recently, there have been reports indicating the double pathogenic variants in the same patient, as in a large pedigree of 33 patients harboring p.Cys634/p.Asp707Glu, with a probably modifying effect in the age of onset and disease severity (103). In addition, investigators have described that the compound p.Cys634Tyr/p.Val292Met transmutation was associated with a more aggressive disease than either p.Cys634Tyr or p.Val292Met variants, indicating a synergic effect of compound nonsynonymous germline *RET* pathogenic variants (104). A novel germline *RET* variant, Ser409Tyr, likely pathogenic, has also been recently reported (105). Notably, a Chinese group reported the first preimplantation genetic diagnosis in a patient harboring a *RET* pathogenic variant (p.Cys634Tyr); one healthy baby was born after 38 gestational weeks (106).

Of note, a rare case of an Arabic patient with bilateral PHEO and a ganglioneuroma component associated with *MEN2A* has been reported in Morocco (107).

Conclusion

Molecular epidemiology evaluates the influence of genetic risk factors in the etiology, diagnosis, and prevention of a genetic disease. The increasing number of patients and geographies studied has demonstrated heterogeneity on the disease presentation even among gene carriers of the same kindred. This heterogeneity justifies a global effort to describe families with *MEN2* to help on clarifying the genetic or environmental circumstances that might impact on disease phenotypes. Here, we show that a large spectrum of *RET* variants is observed worldwide. The codon 634 variants seem to be the most prevalent (Fig. 2 and Table 2), but there are differences in the type of amino acid exchanges among countries and in the frequencies of the other *RET* codon variants.

Some hypotheses may be postulated to explain the variability in the *RET* variant profiles among the countries. Studies using haplotype analysis or pedigree linkage have demonstrated that some pathogenic *RET* variants have been transmitted to offspring for centuries,

Figure 2

Relative frequency of p.Cys634 variants among all *RET* carriers identified in the respective countries. NA, data not available or insufficient data to calculate the frequency.

explaining some local prevalence due to a founder effect. An important drawback of these observations resides in the limited amount or lack of data from many countries, which was somewhat disappointing considering that the causative role of *RET* germline variants in MEN2 syndrome has been demonstrated almost 30 years ago. Moreover, the reports from several countries used different sequence techniques over time; in addition, at the beginning, the goal was on analyzing only *RET* exons 10, 11, and 16; after some years, researchers have extended the search to exons 5, 8, 8–16. Finally, it is important to call attention that the recommendation for the molecular diagnosis of the *RET* has changed from clinically affected MEN2 patients in the early years to all patients with MTC and PHEO nowadays. These changes over time might have resulted in different distribution of *RET* variants. Hopefully, the advent of next-generation sequencing and comprehensive analysis of the *RET* exome, or the entire locus, extended to a larger number of MEN2 patients, shed light on the geographic distribution of *RET* variants and environmental circumstances that might affect genotype–phenotype correlations.

Declaration of interest

A L M has served as principal investigator in multicenter studies for Astra-Zeneca, Exelixis, and Lilly within the past 2 years. R M B M has nothing to disclose.

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