Multiple endocrine neoplasia type 2 syndromes (MEN 2): results from the ItaMEN network analysis on the prevalence of different genotypes and phenotypes

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Abstract

Objective: Multiple endocrine neoplasia type 2 (MEN 2) is a genetic disease characterized by medullary thyroid carcinoma (MTC) associated (MEN 2A and 2B) or not familial MTC (FMTC) with other endocrine neoplasia due to germline RET gene mutations. The prevalence of these rare genetic diseases and their corresponding RET mutations are unknown due to the small size of the study population.

Methods: We collected data on germline RET mutations of 250 families with hereditary MTC followed in 20 different Italian centres.

Results and conclusions: The most frequent RET amino acid substitution was Val804Met (19.6%) followed by Cys634Arg (13.6%). A total of 40 different germline RET mutations were present. Six families (2.4%) were negative for germline RET mutations. The comparison of the prevalence of RET germline mutations in the present study with those published by other European studies showed a higher prevalence of Val804Met and Ser891Ala mutations and a lower prevalence of Leu790Phe and Tyr791Phe (P < 0.0001). A statistically significant higher prevalence of mutations affecting non-cysteine codons was also found (P < 0.0001).

Furthermore, the phenotype data collection showed an unexpected higher prevalence of FMTC (57.6%) with respect to other MEN 2 syndromes (34% MEN 2A and 6.8% of MEN 2B). In conclusion, we observed a statistically significant different pattern of RET mutations in Italian MEN 2 families with respect to other European studies and a higher prevalence of FMTC phenotype. The different ethnic origins of the patients and the particular attention given to analysing apparently sporadic MTC for RET germline mutations may explain these findings.

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Introduction

The human RET gene maps on 10q11.2, and is composed of 21 exons with a size of about 55 kb (1). The RET gene encodes a tyrosine kinase transmembrane receptor (2) characterized by three different domains: the extracellular domain, the transmembrane domain, and the intracellular tyrosine kinase domain. The RET gene is expressed in a variety of neuronal cell lineages including thyroid C cells and adrenal medulla (3).
In 1993, independent groups discovered that germline point mutations of the RET proto-oncogene are causative events in multiple endocrine neoplasia type 2 (MEN 2) (4–6). MEN 2 are autosomal dominant inherited syndromes characterized by the presence of medullary thyroid carcinoma (MTC) in 100% of cases, associated or not familial MTC (FMTC) with other endocrine tumors like pheochromocytoma (PHEO) and/or parathyroid adenomas (hyperPTH) in MEN 2A or only PHEO in MEN 2B (7). Other non-endocrine diseases are typically present in MEN 2B (mucosal neuromas, megacolon, corneal nerve hypertrofia, and habitus marphanoid) (8, 9) and occasionally in MEN 2A (cutaneous lichen amyloidosis) (10, 11). Finally, Hirschsprung’s disease can be associated with both MEN 2A and MEN 2B (12, 13).

The incidence of the MEN 2 syndromes is unknown. Because MTC represents only 5–10% of all thyroid tumors, being sporadic in 75% of cases and familial in 25% of cases, the prevalence of MEN 2 is ~1–3% of all thyroid malignant tumors. Since thyroid tumors represent only 1% of all human cancers (14), it is evident that MEN 2 is a very rare disease, and only cooperative studies have been able to include an appropriate number of affected subjects and families to accurately correlate RET mutation and MEN 2 syndrome (15).

The number and type of RET point mutations associated with MEN 2 have been growing over the last 10 years, especially after the introduction of RET genetic screening in the work-up of all patients with MTC (16, 17). Several studies demonstrated the presence of a RET germline mutation in 5–10% of apparently sporadic cases, mainly located in non-cysteine codons (18–20). Owing to the rarity of the MEN 2 syndromes, the prevalence of the different RET mutations is not well defined, and it is not clear whether specific mutations are more prevalent in distinct geographic areas (21–23). So far, there are only four published European studies collecting data from several centres in which the prevalence and distribution of different RET mutations have been investigated (2, 23–25).

The aims of the present study were i) to collect RET genetic screening data from as many Italian families as possible affected by MEN 2/FMTC, ii) to analyze the distribution and frequency of RET mutations in this study of families, and iii) to compare the type and prevalence of RET mutations to those reported in previous studies of European families of affected individuals.

Patients and methods

The majority of the Italian centres involved in the diagnosis and treatment of thyroid carcinoma were invited to complete a form containing the following information for any patient with hereditary or sporadic MTC analyzed by RET genetic screening: i) name and surname of the index case, ii) other surnames in the same family to avoid multiple inclusion of the same family members, iii) type of RET mutation, and iv) MEN 2 phenotype according to the definitions reported in the recent American Thyroid Association guidelines on MTC management (26).

A database recording the collected information was organized at Pisa University Hospital, which is a national referral centre for the diagnosis and treatment of thyroid cancer and where 103 MTC families are currently followed. Only Italian patients were included in the study. The study was approved by the Internal Reviewing Board of the Department of Endocrinology and Metabolism in Pisa.

All patients signed an informed consent to genetic analysis.

We also ascertained that in each centre, the RET mutation analysis had been performed according to
the same procedure. Briefly, RET gene mutation screening was performed on genomic DNA extracted from the blood of patients with hereditary or sporadic MTC, and analyzed by sequence analysis as previously reported (18).

As inclusion criterion, the centres were asked to confirm that they routinely investigated for germline mutations, all cases of MTC, also those apparently sporadic, and that at least the six more frequently affected exons (i.e. 10–11, 13–16) were commonly studied.

**Statistical analysis**

Data analysis was performed using StatView 4.5 software (Abacus Concepts Inc., Berkley, CA, USA). We adopted the $\chi^2$ test to analyse data collected from participating centres. A $P$ value $<$ 0.05 was regarded to be statistically significant.

**Results**

We collected clinical and molecular data on hereditary MTC patients from 20 different Italian centres with a total of 250 families. In a few cases, different members of the same family were found to be followed in multiple centres: nevertheless, these families were considered only once.

Overall, 40 different germline RET mutations were identified. However, six families (6/250, 2.4%) were negative for germline mutations, despite a more accurate analysis covering the entire RET coding sequence. The analysis of the prevalence of the different RET mutations showed that the most frequent RET amino acid substitution was Val804Met (49/250, 19.6%) followed by Cys634Arg (34/250, 13.6%; Fig. 1). In addition, codon 634 at exon 11 was the most frequently altered codon ($n$ = 87, 34.8%; Fig. 2), and consequently, exon 11 was the most frequently altered exon.

Phenotypes were defined in all centres following the same criteria (26). According to this classification, 85/250 (34%) kindred were classified as MEN 2A, 17/250 (6.8%) as MEN 2B, and 144/250 (57.6%) as FMTC. In 3/250 (1.2%) cases (Fig. 3), the presence of a germline RET mutation was not correlated with a clinically developed MTC, and the RET genetic screening was performed for the evidence of hypercalcitoninemia and C cell hyperplasia in two kindred (Fig. 3, families C and D) and for Hirschsprung’s disease in one (Fig. 3, family A). In 1/250 (0.4%) cases (Fig. 3, family B), the mutation (Arg694Gln) was detected during a random screening for RET polymorphisms in the general population, and was not associated with MTC or hypercalcitoninemia, in keeping with its lack of in vitro-transforming activity, as previously reported (40); these four kindred were classified as ‘Others’ and the corresponding mutations as variants of unknown significance (VUS). These four families and their corresponding mutations were not considered in the following statistical evaluation. As shown in Table 1, the majority of MEN 2A cases were associated with mutations at codon 634, while MEN 2B was exclusively associated with the mutation at codon 918. Conversely, the genotype–phenotype correlation in FMTC and others was much more heterogeneous in terms of involved codons. As previously observed (16, 18), the majority of these two last groups of patients harbourd a RET germline mutation in a non-cysteine codon. Among the six families without a RET germline mutation, we distinguished five FMTC and one MEN 2A kindred.

As shown in Table 2, the comparison of the prevalence of RET germline mutations in the present study with those found in studies published by other European Study Groups showed a statistically significant difference ($P < 0.0001$). In particular, there was a significantly higher prevalence of Val804Met ($P < 0.0001$) and Ser891Ala ($P = 0.0012$) mutations in the Italian study, which were relatively rare in both EUROMEN and German groups. Conversely, a lower

**Figure 3** Pedigrees of families classified as ‘Others’. Cases with reported age have been tested for the RET mutation and RET-positive cases are marked with an asterisk. Family A: only the 8-year-old child was affected with Hirschsprung’s disease; neither MTC nor elevated serum calcitonin (CT) was found in the index case and family members; Family B: RET-positive cases found by screening of normal persons; neither MTC nor elevated serum CT was found in this family; Families C and D: elevated serum CT and C cell hyperplasia in cases with RET mutation but not in the other members of the families.

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**Table 1** Prevalence of MEN 2 syndromes in Italy

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Number of Families</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEN 2A</td>
<td>85</td>
<td>34.0%</td>
</tr>
<tr>
<td>MEN 2B</td>
<td>17</td>
<td>6.8%</td>
</tr>
<tr>
<td>FMTC</td>
<td>144</td>
<td>57.6%</td>
</tr>
</tbody>
</table>

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**Table 2** Comparison of RET germline mutation prevalence in the present study with other European Study Groups

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Present Study</th>
<th>EUROMEN</th>
<th>German Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Val804Met</td>
<td>49/250 (19.6%)</td>
<td>17/100 (17.0%)</td>
<td>8/100 (8.0%)</td>
</tr>
<tr>
<td>Ser891Ala</td>
<td>34/250 (13.6%)</td>
<td>2/100 (2.0%)</td>
<td>2/100 (2.0%)</td>
</tr>
</tbody>
</table>

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*This family has been published by Orgiana et al. (40)
The prevalence of mutations Leu790Phe (P = 0.0017) and Tyr791Phe (P = 0.0002) was observed in the present study, especially when compared with the German study. A statistically significant difference was also observed in the prevalence of Met918, which was much higher in the German study with respect to the others (P = 0.0032) and of Cys634, which was much higher in the EUROMEN study (P < 0.0001).

We also observed a high prevalence of several mutations affecting non-cysteine codons, some of which were never reported before. As shown in Table 3, when we compared the percentage of RET mutations affecting cysteine and non-cysteine codons, we found a statistically significant difference among the three groups with a higher prevalence of mutations affecting non-cysteine codons in our study (P < 0.0001).

### Discussion

MEN 2 syndromes are rare, and affected patients are usually referred to specialized centres. In the present study, the majority of the Italian referral centres replied to an invitation to share data on familial MTC and the data collected, which correspond to the largest European study available to date, clearly show a widespread distribution of cases at a national level. We are aware that this survey is not complete due to the fact that a few centres did not reply to our invitation and because a few MEN 2 families may be followed in peripheral centres which could not be reached by our first call for families. Nevertheless, the majority of the Italian referral centres have been contacted and, with a few exceptions, they replied to our invitation.
Table 2 Prevalence of RET germline mutations in hereditary medullary thyroid carcinoma: a comparison among three different European studies.

<table>
<thead>
<tr>
<th>RET mutation</th>
<th>Germany (n=141)</th>
<th>EUOMEN (n=145)</th>
<th>Italy (n=246)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Met918</td>
<td>21 (15)</td>
<td>4 (2.8)</td>
<td>20 (8.1)</td>
</tr>
<tr>
<td>Cys634</td>
<td>57 (40)</td>
<td>98 (67.6)</td>
<td>86 (34.9)</td>
</tr>
<tr>
<td>Asp631</td>
<td>1 (0.7)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cys630</td>
<td>1 (0.7)</td>
<td>1 (0.8)</td>
<td>4 (1.6)</td>
</tr>
<tr>
<td>Cys620</td>
<td>10 (7)</td>
<td>10 (6.9)</td>
<td>9 (3.6)</td>
</tr>
<tr>
<td>Cys618</td>
<td>7 (5)</td>
<td>10 (6.9)</td>
<td>15 (6.0)</td>
</tr>
<tr>
<td>Cys611</td>
<td>2 (1.4)</td>
<td>4 (2.8)</td>
<td>1 (0.4)</td>
</tr>
<tr>
<td>Cys609</td>
<td>1 (0.7)</td>
<td>1 (0.8)</td>
<td>6 (2.4)</td>
</tr>
<tr>
<td>Leu790</td>
<td>17 (12)</td>
<td>7 (4.8)</td>
<td>8 (3.2)</td>
</tr>
<tr>
<td>Tyr791</td>
<td>10 (7)</td>
<td>3 (2.1)</td>
<td>1 (0.4)</td>
</tr>
<tr>
<td>Val804</td>
<td>9 (6.4)</td>
<td>3 (2.1)</td>
<td>52 (21.1)</td>
</tr>
<tr>
<td>Ser891</td>
<td>3 (2.2)</td>
<td>3 (2.1)</td>
<td>23 (9.3)</td>
</tr>
<tr>
<td>Glu768</td>
<td>2 (1.4)</td>
<td>1 (0.8)</td>
<td>9 (3.6)</td>
</tr>
<tr>
<td>Ala883</td>
<td>0</td>
<td>0</td>
<td>1 (0.4)</td>
</tr>
<tr>
<td>Cys515</td>
<td>0</td>
<td>0</td>
<td>1 (0.4)</td>
</tr>
<tr>
<td>Lys666</td>
<td>0</td>
<td>0</td>
<td>1 (0.4)</td>
</tr>
<tr>
<td>Met918</td>
<td>0</td>
<td>0</td>
<td>1 (0.4)</td>
</tr>
<tr>
<td>Ser904</td>
<td>0</td>
<td>0</td>
<td>1 (0.4)</td>
</tr>
<tr>
<td>Thr338</td>
<td>0</td>
<td>0</td>
<td>1 (0.4)</td>
</tr>
</tbody>
</table>

*VUS have been excluded.

Although we cannot calculate the prevalence of MEN 2 in Italy, which indeed was not the aim of this study, the study was large enough to show the prevalence and the distribution of both different RET gene mutations and different MEN 2 phenotypes (i.e. MEN 2A, 2B, and FMTC). We observed an overall different distribution of several germline RET mutations, and in particular, we found that Val804Met, which is localized in exon 14, is the most frequent RET mutation in Italy. A high prevalence of Val804Met mutation was firstly reported in a Sardinian study, and this finding was interpreted as a possible consequence of the remarkable influence of genetic drift and of the founder effect in the history of this population living isolated for several centuries (21). As a matter of fact, several other genetic diseases have shown a different (and often higher) prevalence in Sardinia with respect to other Italian regions (27–29). However, this hypothesis was not confirmed by the present study, showing an overall high prevalence of Val804Met mutation in Italy with respect to other European countries (16, 23, 24, 30). Moreover, in our study, the presence of a founder effect was excluded, because the analysis of the pattern of the RET gene single-nucleotide polymorphisms in index cases harboring the Val804Met mutation was different in different patients (data not shown).

With a few exceptions (31–34), RET Val804Met mutation is usually correlated with the FMTC phenotype, and in the majority of cases, the aggressiveness of the thyroid tumor is relatively low. However, it has been demonstrated that this mutation is refractory to many tyrosine kinase inhibitors (TKI) (35), which nowadays are considered the best target therapy under development (36). It is of concern that if Val804Met mutation is so prevalent, some of these patients might develop an aggressive form with no possibility of being treated with TKI.

When comparing the RET mutations prevalence in our study with those reported in other European study (2, 16, 18, 23–25, 31), we also observed a higher prevalence of Ser891Ala mutation in exon 15. We found that this mutation was mainly present, although not exclusively, in a well-defined area of Northern Italy, and the possibility of a founder effect is under investigation (37). Conversely, a lower prevalence of both Leu790Phe and Tyr791Phe was evident especially when the comparison was performed between the German study and ours. It is conceivable that different ethnic origins of the patients included in the different series may explain these statistically significant differences.

It is worth noting that a certain number of RET mutations described in the present study have never been reported before (http://arup.utah.edu/database/MEN2/MEN2_welcome.php). Some of these mutations have been found in patients affected with MTC, while others were found in subjects screened for other reasons (generally hypercalcitoninemia). While some of them have been studied for their transforming ability (38–40) others, such as Lys666Met, Met848Thr, Met918Val, Ser904Phe, Thr338Ile, and Val648Isole, have not been investigated. Since at least one of these mutations (Arg654Gln) was proven to be devoid of transforming activity (40), caution should be exercised in the interpretation of the actual pathogenic role of these rare mutations. Nevertheless, we previously reported a germline RET mutation associated with MTC in two homozygous brothers but not in four other heterozygous relatives (38). Although the transforming activity of this RET mutation was proven, we can speculate that there are RET mutations with a very low transforming activity, not necessarily observed in ‘in vitro studies’, which require a long period of time or a second reinforcing mutation on the other allele to trigger the tumoral transformation. As a matter of fact,

Table 3 Comparison of prevalence of cysteine and non-cysteine RET mutations in the three different European studies.

<table>
<thead>
<tr>
<th>Study</th>
<th>Cysteine</th>
<th>Non-cysteine</th>
<th>P</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>German Austria (n=141)</td>
<td>56</td>
<td>44</td>
<td></td>
<td>Frank-Raue et al. (1996) (25)</td>
</tr>
<tr>
<td>EuroMen (n=145)</td>
<td>86</td>
<td>14</td>
<td>P&lt;0.0001</td>
<td>Machens et al. (2003) (24)</td>
</tr>
<tr>
<td>ItaMEN (n=240)*</td>
<td>50.8</td>
<td>49.2</td>
<td></td>
<td>Present study</td>
</tr>
</tbody>
</table>

*The six RET-negative families and the four ‘Others’ families have been excluded.
in the majority of cases, the kindred affected by these rare RET mutations are usually very small or even restricted to a single-index case found with a germline RET mutation. It must be remembered that one of the inclusion criteria in this study was that all MTC, including those presenting as sporadic form from a clinical and anamnestic point of view, should have been screened for RET mutations in the six most commonly altered exons. Furthermore, in our clinical practice, it is common that when no mutations are found in the most commonly altered codons, other exons are investigated thus increasing the possibility of finding new mutations outside the hot spots. This very intensive search for RET mutations might surface rare mutations which are mainly located in the non-cysteine RET coding codons. To reveal the risk profile of these uncommon RET mutations, a larger recruitment of family members is desirable to better correlate genotype and phenotype. In vitro studies of the tumoral transforming activity are also necessary. At present, these mutations could be considered as allelic variants (41, 42).

While the phenotype of MEN 2A and 2B is relatively easy to be defined because based on the positive family history and/or the association with other endocrine and non-endocrine diseases (7, 15, 26), the definition of FMTC is more challenging because it may be thought that the other endocrine neoplasms are not yet developed at the time of FMTC diagnosis. The problem is negligible in large multigenerational families with several members affected by only MTC, while in smaller families with few affected members caution should be posed as there is the risk of underestimating the possibility of later developing PHEO. This problem is of particular relevance because, at least in our study, the prevalence of FMTC is much higher than that reported before and the FMTC phenotype is largely prevalent with respect to the others (16, 25, 33). To our knowledge, this high prevalence of FMTC (56.6%) has never been reported before and, in our opinion, this finding is related to both the more recent introduction of the RET screening in the work-up of apparently sporadic MTC and the more extensive search for RET mutations in non-hot spot regions of the gene (18, 32, 43).

In conclusion, this study allowed us to have a more comprehensive vision of the RET mutations and MEN 2 phenotypes distribution and prevalence in Italy. To our knowledge, this is the first and unique study collecting data from the majority of Italian centres involved in the diagnosis and treatment of thyroid cancer. We observed several statistically significant and peculiar differences in the prevalence of specific RET mutations with respect to other European studies. These differences can be due both to the different ethnical origins of the patients belonging to the different studies and also to the particular attention in looking for germline RET mutations also in apparently sporadic MTC which, if they turn out to be hereditary, are more frequently associated with non-cysteine RET mutations. These latter hypotheses can also explain the other relevant result of this study showing that FMTC is the most prevalent MEN 2 phenotype, at least in Italy.

Declarations of interest

All the authors disclose any financial and personal relationships with people or organisations that could inappropriately influence their work.

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References

Prevalence of MEN 2 syndromes in Italy


Identification of a novel point mutation in the RET gene (Ala883Thr), which is associated with medullary thyroid carcinoma phenotype only in homozygous condition. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 5823–5827.


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