Multiple Endocrine Neoplasia Type 2A: Genetics and Prophylaxis

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ABSTRACT

MEN 2, which includes MEN2A, MEN 2B, and FMTC is caused by autosomal dominant gain-of-function mutations of RET proto-oncogene. MEN2A is the most frequent form. This syndrome is characterized by a medullary thyroid carcinoma (MTC) and/or pheochromocytoma (PHEO) and/or multiple tumors of the parathyroid glands. Early prophylactic total thyroidectomy before the development of MTC is currently the only curative treatment. The aims of this study were to characterize point mutations in RET proto-oncogene in the family index case, to identify and assess the effectiveness of the management of the index case relatives who have presented the RET proto-oncogene mutation and to search the existence of a possible correlation between the phenotype and genotype among the members of this MEN 2A family. Our work focused on a family of MEN 2A with an index case with MTC and pheochromocytoma, and 13 1st degree relatives. Exons 8, 9, 10, 11, 13, 14, 15 and 16 of the RET proto-oncogene were analysed by PCR/sequencing. We characterized the exon 11 RET proto-oncogene germline mutation C634Y in the index case MEN 2A and in 7 of his 13 1st degree relatives. The haplotype G691S / S904S of RET in the homozygous state was found in the relatives who presented the mutation suggesting that this haplotype have a modifying effect on the age of onset of MTC in MEN2A. In our patients, the C634Y mutation was significantly associated with the presence of pheochromocytoma, which is consistent with the literature data. The C634Y is characterized by a particularly aggressiveness, thyroidectomy is then recommended before the age 5 years. All patients described in this report have undergone thyroidectomy (6 cases), removal of PHEO (only 4 cases).

Keywords: MEN2A, RET proto-oncogene, mutation C634Y, prophylactic thyroidectomy, Pheochromocytoma ablation.

INTRODUCTION

Multiple endocrine neoplasia type 2 (MEN 2) is an inherited multiglandular disorder, with an estimated prevalence of 2.5 per 100,000 in the general population in Europe. MEN 2 syndrome includes three clinically different inherited syndromes.

-MEN2A associating medullary thyroid carcinoma (MTC) and/or pheochromocytoma (PHEO) and/or multiple tumors of the parathyroid glands.

-MEN2B characterized by MTC, pheochromocytoma, Marfan type dysmorphism and ganglioneuromatosis submucosa and digestive.

-Familial medullary thyroid carcinoma (FMTC), affected family members develop MTC only.

These three phenotypic variants have as a constant the presence of an MTC with high penetrance. Affected individuals develop early primary C-cells hyperplasia (CCH) which progresses to invasive MTC. However, the C cells hyperplasia is a primary neoplastic lesion.

Serum calcitonin (CT) is an MTC recognized biomarker. Its base elevation associated with its increase after injection of pentagastrin (Pg test) is pathognomon of MTC.

These three subtypes of MEN 2 differ in their incidence, age of onset, MTC aggressiveness, genetics, association with other diseases, and prognosis.

MEN 2A, is the most common form (60% of MEN 2). It is characterized by MTC, pheochromocytoma, and hyperparathyroidism in one patient, or the presence of two or more tumors in multiple members of the same family.

The MEN 2A MTC (frequency over 90%) is not always the first manifestation of the disease. It then associates a PHEO in 20% to 50% of cases. These are usually bilateral sometimes with a dissociation time. It can be completely asymptomatic (60% of cases) and constitute by their presence a real threat of death by a fatal cardiogenic shock. Their research is absolutely indispensable in the presence of MTC because their treatment must precede that of the MTC. Primary hyperparathyroidism (HPT1) is in 15-30% of cases practically always asymptomatic.

In some cases, MEN 2A can be associated with paraneoplastic syndromes such as a rash pruritic sitting in the upper back, lichen amyloid or “notalgia” incriminating a keratin deposit. A skin biopsy at their level reveals amyloid deposits at the dermal-epidermal junction or a thickening of the corneal nerve fibers, previously considered characteristic.
MEN 2B, but can be observed in some cases of MEN2A. A motor diarrhea, flush and an Hirschsprung syndrome can also be seen. About 15% of MTC are associated with ectopic ACTH production and Cushing’s syndrome. 27

The three MEN 2 variants are linked to activating RET proto-oncogene germline mutations and are inherited in an autosomal dominant pattern.

RET proto-oncogene is located on the long arm of chromosome 10 at 10q11.2, and spans about 60kb. Its coding region is divided into 21 exons28,30 and encodes a tyrosine kinase transmembrane receptor, expressed in several developing tissues and mainly in those derived from the neural crest.

RET appears to transduce growth and differentiation signals in several developing tissues.31

RET proto-oncogene germline mutations have been reported in 97% of MEN 2A cases and are crucial for the onset and the progression of MTC.

These mutations are mostly localized in exon 11 at codon 634, in exon 10 at codons 609-611-618-620 and other mutations located in exons 8, 10, 11 and 13-15 have been also observed.

Actually, especially after the introduction of RET genetic screening in the work-up of all patients with MTC, there is a change in the spectrum of detecting RET mutations and an increase of diagnosed cases from 10% to 39% of the families with mutations in exon 13-15, so called “rare mutations”.32,33

Characterization of RET proto-oncogene germline mutations, has made possible genetic testing for MEN 2. The efficiency of this screening justifies it to be advocates for children and adults or a preventative treatment would be possible.

The aims of this study were to characterize point mutations in RET proto-oncogene in an index case and his relatives, to Identify and assess the effectiveness of the management of the index case relatives who have presented the RET proto-oncogene mutation and to search the existence of a possible correlation between the phenotype and genotype among the members of this MEN 2A family.

PATIENTS AND METHODS

A 33-year-old man, the fourth in a family of seven brothers and sisters, married with five children alive and well has consulted for a permanent hypertension.

The interrogation and clinical examination revealed: The presence of a thyroid nodule, diarrhea and notalgia. In his family history, there is the notion of the death of his mother at a young age in a total thyroidectomy on nodular goiter and hypertension (Fig.1.). A number of investigations were performed for our patients as a blood calcitonin, which reverted to 106 pg/ml, urinary metanephrines 6.30 µmol/24 h. Serum calcium, PTH and CEA were normal. The MIBG scintigraphy revealed bilateral adrenal tumor and cervical ultrasound showed a thyroid nodule.

Basal serum calcitonin (bCT), PTH and CEA levels were assessed by immune - chemiluminescent/magnetic particle (ICMA)(Abbott Diagnostics assay). Determination of urinary metanephrine was performed by high-performance liquid chromatography (CLHP) and serum calcium by arsenaso III color. Bilateral PHEO ablation followed by total thyroidectomy was done and MTC and PHEO were confirmed by histopathology of the surgically removed tumors.34

The relatively young age of the discovery of the PHEO and the MTC suggested that it is about a potential MEN2A syndrome and thus the analysis of RET proto-oncogene exons: 8.9.10.11.13.14.15 and 16 has been performed.

After extracting DNA from peripheral blood leukocytes using the standard salting out method35, the search for mutations in the RET proto-oncogene was carried out by PCR/sequencing as previously reported.36

DNA sequencing reactions were performed using the Big Dye Terminator V3.1 Cycle Sequencing Kit and sequenced on an ABI PRISM 3130 Automated Sequencer (Applied Biosystems, Foster City, CA, USA). All RET exons were sequenced using both forward and reverse primers.

RESULTS

We have identified, in the index case, the RET proto-oncogene, exon 11 germline mutation C634Y a transition G > A at nucleotide level c2096 resulting in TGC conversion to CGC, substituting cysteine to arginine, in the heterozygous state (Fig.2.) and the G691S polymorphisms (exon11) (Fig.3.) S904S (exon15) in the heterozygous state.

Figure 2: Partial DNA sequence for exon 11 of RET proto-oncogene, showing c. 2096G > A (C634Y) heterozygous variant found in the proband

![Figure 1: The Index Case Family Pedigree](image)
Figure 3: Partial DNA sequence for exon 11 of RET proto-oncogene, showing **G691S** heterozygous variant found in the proband.

Then the screening of the C634Y mutation of RET has been offered to all the relatives (Fig.4.) recommendation 7 of the revised ATA Guidelines. 

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**Figure 4:** The index case family pedigree after the genetic screening of the C634Y mutation.

<table>
<thead>
<tr>
<th>Number of Relatives</th>
<th>Diagnosis</th>
<th>Mutation</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 relatives</td>
<td>NEM2A</td>
<td>C634Y</td>
<td>58.33 %</td>
</tr>
<tr>
<td>5 relatives</td>
<td>Unharmed</td>
<td>-</td>
<td>48.33 %</td>
</tr>
</tbody>
</table>

Table 1: Frequency of Mutation C634Y in Relatives

In relatives, Exon 11 RET proto-oncogene analysis identified the C634Y exon 11 mutation in the heterozygous state with a frequency of 58.33% (Table 1).

The screening of the other exons in search of polymorphisms showed that the latter were identical to those of the index case but on the homozygous state (Fig.5) (Fig.6).

**Figure 5:** Partial DNA sequence for exon 15 of RET proto-oncogene, showing G691S homozygous variant found in the relatives.

**Figure 6:** Partial DNA sequence for exon 15 of RET proto-oncogene, showing S904S homozygous variant found in the proband (TCC/TCG)

The average age of the discovery of the mutation in relatives was 18.57 ± 14.87 years (Tabl.2) with extremes of 4 and 40 years.

**RET** mutated relatives were submitted to clinical and biochemical examination to ascertain thyroid, parathyroid, and adrenal gland involvement.

Screening for PHEO and hyperparathyroidism was performed both at diagnosis and annually during the follow-up for a possible prophylactic taking load.

Sixty-six point sixty-six percent (66.66%) of the MEN 2A index case relatives showed a high rate of calcitonin before surgery. And 50% of them have seen the persistence of high rates following surgery (Tableau.2).

The measurement of urinary catecholamine incomes positive in 42% of the relatives who made the determination.

All the relatives were recommended for a preventive total thyroidectomy and pheochromocytoma ablation (Table 2).
### Table 2: Biological, Genetic and Clinical Features of the 7 Relatives Patients

<table>
<thead>
<tr>
<th>Index Case Relatives</th>
<th>Age at Diagnosis by Years (Mean ± SD)</th>
<th>Mutation</th>
<th>CT (pg/ml) Before Surgery</th>
<th>CT after Thyroidectomy Surgery</th>
<th>Urinary Meta Nephrine (µmol/24 h)</th>
<th>Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nephew (III16)</td>
<td>4 years</td>
<td>C634Y</td>
<td>11</td>
<td>&lt;2</td>
<td>0.37</td>
<td>Thyroidectomized</td>
</tr>
<tr>
<td>Son (III-11)</td>
<td>4 years</td>
<td>C634Y</td>
<td>756</td>
<td>760/766/478/423</td>
<td>0.30</td>
<td>Thyroidectomized</td>
</tr>
<tr>
<td>Daughter (III-10)</td>
<td>10 years</td>
<td>C634Y</td>
<td>5</td>
<td>&lt;2</td>
<td>0.25</td>
<td>Thyroidectomized</td>
</tr>
<tr>
<td>Nephew (III-13)</td>
<td>11 years</td>
<td>C634Y</td>
<td>11</td>
<td>&lt;2</td>
<td>0.20</td>
<td>Thyroidectomized</td>
</tr>
<tr>
<td>Younger brother (II-12)</td>
<td>27 years</td>
<td>C634Y</td>
<td>86</td>
<td>106/429</td>
<td>4.50</td>
<td>Thyroidectomized+ pheochromocytoma ablation</td>
</tr>
<tr>
<td>Sister (III-3)</td>
<td>40 years</td>
<td>C634Y</td>
<td>558.82/430.8</td>
<td>116/76.6/154</td>
<td>11.03</td>
<td>Thyroidectomized+ pheochromocytoma ablation</td>
</tr>
<tr>
<td>Sister (II-9)</td>
<td>34 years</td>
<td>C634Y</td>
<td>4.87</td>
<td>&lt;2</td>
<td>3.22</td>
<td>Thyroidectomized+ pheochromocytoma ablation</td>
</tr>
</tbody>
</table>

**Serum (pg/ml):**
- Calcium, parathyroid hormone, Serum CEA were Normal
- CT (pg/ml): Normal value 8.4 pg / ml in men and 5 pg/ml in women
- Urinary metanephrine: Normal value = 0.20-1 µmol/24 h
DISCUSSION

Genetic analysis of the RET proto-oncogene has become indispensable in the management of MEN2 syndrome. It allows early surgical indications in genetically predisposed. During this work, we have identified a MEN 2A family from Constantine and characterized the RET proto-oncogene, exon 11 germline mutation C634Y in the heterozygous state. Among the germline mutations of cysteine codon, mutations at codon 634 position are the most highly transforming, more common and occur in ~85% of NEM 2A families, also found in FMTC (30%).

The C634Y represents approximately 26% of all mutations at this codon while the C634R mutation (Cys>Arg) represents more than 50%.

The C634Y mutation was first described in Morocco in MEN 2A. Sánchez and al. In a study, showed that the most frequent RET mutation in MEN 2A Spanish families was the C634Y also in Japanese and Chinese.

In MEN 2A populations, the C634Y incidence is high relatively to C634R. The RET proto-oncogene mutation analyses in French hereditary MEN2A revealed that the most frequent mutations in this population were the C634R and C634Y.

This mutation is also present in Caucasians and Americans in which the C634R mutation is the most common.

Exon 11 codon 634 mutation causes ligand-independent dimerization of receptor molecules, enhanced phosphorylation of intracellular substrates and cell transformation.

The mutation of a cysteine residue leaves the unpaired partner cysteine free to form intermolecular disulfide bonds, there by generating covalent receptor dimers that display the constitutive activity.

Among the relatives, 7 (58.33%) C634Y carriers have been identified and these patients were examined for the MTC, the PHEO and HPT for possible taking prophylactic load. And 5 of the relatives (48.33%) will not be followed.

The G691S/S904S RET haplotype was present in the homozygous state in relatives. Several studies suggested that this haplotype appears to influence the age of onset of MTC in patients with the MEN 2A syndrome and patients homozygous for these polymorphisms were, on average, diagnosed with MTC 10 years earlier than patients sharing this haplotype in the heterozygous or wild state.

The G691S polymorphism is considered as a functional polymorphism. The change of the aminoacid G → S (glycine to serine) in the RET protein sequence creates a new phosphorylation site, which affects the downstream signaling. It could also lead to a change in the secondary structure of RET, affecting the flexibility and accessibility of protein solvents. The S904S SNP does not lead to an aminoacid change but, appears to be related to the SNP G691S due to a founding effect.

Fifty percent (50%) of the relatives have seen the persistence of high rates of Calcitonin (CT) following surgery. As a part of the MTC, the preoperative CT assay must allow to propose an adequate surgery immediately. Moreover the results of the surgical recovery are assessed on the postoperative CT dosage.

The measurement of urinary methoxyamines income was positive in 42% of the relatives. Indeed PHEO diagnosis is in first-line a biological diagnosis based on the rise of urinary or plasma methoxyamines.

For mutations at codon 634, the risk of developing unilateral or bilateral pheochromocytoma is high. It appears in 57% of patients, and 15–30% of codon 634 mutations carriers will develop hyperparathyroidism.

Patients with C634YRET mutation should be screened periodically from 8 years of age for hyperparathyroidism by measuring serum calcium and parathyroid hormone.

In MEN2, it has shown that specific RET mutations may provide the phenotypic expression of the disease, the MTC aggressiveness, and can provide information on MTC prognosis, could guide prophylactic thyroidectomy and the screening of PHEO and HPT.

Phenotypic analysis of the MEN 2A family has shown that: In one case the MTC has been preceded by 4 years the occurrence of PHEO. In 3 cases, the discovery of the MTC and PHEO was simultaneous and in 2 cases the MTC was the only endocrine neoplasia present both at diagnosis and during follow-up. A paraneoplastic clinical and biological cushing was observed in the elder sister. Notalgia and diarrhea in the index case and the remaining relative was asymptomatic.

Several studies have shown a strong correlation between the codon 634 mutation and risk of PHEO and/or HPT in affected MEN 2A families. We also observed this association MTC and PHEO in the members of this family.

These PHEO are almost synchronous or metachronous to the MTC. They are the first symptom in 13–27% of individuals with pheochromocytomas and MEN 2A.

Their penetration increases with age, being 25% by age 30 years, 52% by age 50 years, and 88% by age 77 years, almost always benign and are usually bilateral, and confined to the adrenal gland. The tumors are usually associated with diffuse nodular adrenal medullary hyperplasia.

Children with MEN2A and RET codon 634 mutations (ATA-H category) often develop MTC during the first years of life; therefore, annual physical examination, cervical US, and measurement of serum CT levels should begin at 3 years of age.

Because, related and unrelated individuals, with the same germline RET mutations, develop MTC and PHEO at different ages, many authors have suggested that other genetic or epigenetic events can trigger the tumorogenesis including the presence of SNP in RET.
Diarrhea, represents the most frequent systemic manifestation, occurs in affected persons with a plasma calcitonin concentration of more than 10 ng/mL and implies a poor prognosis.66,67

Natalgia was observed in the MEN 2A case index. Indeed the nostalgia in MEN 2A has been observed in some families.20

This lichenoid skin lesion is located over the upper portion of the back and may appear before the onset of MTC68 and is associated in 36% of cases to the mutation at RET 634 codon.15,69

The paraneoplastic Cushing syndrome was observed in 15% of MEN 2A cases. It is associated with ectopic ACTH production.27,70,71

According to the revised American Thyroid Association guidelines for the management of medullary thyroid carcinoma and especially the recommendation that says that the current level C category should be changed to a new category, “high risk” (H) that includes patients with the RET codon C634 mutations and the RET codon A883F mutation.96

The C634Y (ATA-H category) is then characterized by a particular aggressiveness. Thyroidectomy is then recommended before the age 5 years (Recommendation 35 ATA/GTE).72,73

Recommendations for the timing of prophylactic thyroidectomy and the extent of surgery are based upon a model that utilizes these genotype-phenotype.6,74

All patients described in this report have undergone thyroidectomy (7 cases), and PHEO ablation only in 4 cases.

The relatives that having been operated on the basis of a germline RET mutation, C-cell hyperplasia, a premalignant lesion, has been found in one case, microscopic MTC in 2 cases, MTC in 3 cases.

Three patients had lymph node metastases. These results are identical to those found by different studies.55,75,76

In the literature, for mutations located in exon 10 or 11, mainly corresponding to the NEM2A 634 mutation, the prevalence of cancer at the age of five years, is from 50 to 60%, but the possibility of the development of a micro cancer as early as at the age of 15 months has been reported46,77,78,79.

Similarly lymph node involvement is considered rare before the age of ten years.80 In children, the cure at ten years is in 100% after MTC surgery in the absence of lymph node involvement, it drops to 84%, in case of lymph node involvement.81

Conversely, in patients with a MEN2 A phenotype, monitoring should be prolonged, indefinite, yearly to detect the occurrence of phaeochromocytoma. An annual dosage of calcium, supplemented if hypercalcemia by a parathyroid hormone assay will complement the assessment carried out in search of phaeochromocytoma.

The screening for PHEO should begin by age 11 years for children in the ATA-H as saying the ATA Recommendation 37,2 while the European Thyroid Association recommends the beginning of the biochemical screening for phaeochromocytoma and hyperparathyroidism at 8 years of age (Recommendation 7).34

For the timing of prophylactic thyroidectomy and the extent of surgery are based upon a model that utilizes these genotype-phenotype.35

The age of thyroidectomy in patients with the C634Y mutation was not respected because the related screening has been achieved, when the MEN 2A case index was identified, but also for psychological and cultural reasons. It is therefore evident that, although these interventions were scheduled to be prophylactic, over 80% of them have been therapeutic.

In fact, recommendations for thyroidectomy are respected in practice less than 15% of the cases for fear of complications of early surgery.34

CONCLUSION

This work contributed to the development of genetic testing for MEN 2 in Algeria.

This molecular screening has become an essential tool. It allows, very early, to distinguish individuals at risk of carrying the mutation those that are not carriers and which can be excluded from monitoring for the rest of their life. The surgery is an alternative treatment (prophylactic and/or curative).

Through this work, we hope to contribute to the formation of a multidisciplinary team whose nucleus already exists for the development of genetic testing for MEN 2 and the establishment of a national consensus adapted to our country, for the MEN2 prophylactic thyroidectomy.

This will encourage the development of a national policy against this cancer whose care is very expensive.

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