ORIGINAL ARTICLE

Phenotype of the C634Y mutation in the RET proto-oncogene in MEN2A: report of a family

Paula Sánchez Sobrino*, Concepción Páramo Fernández, Pedro Gil Gil, Beatriz Mantiñán Gil, Alberto Pérez Pedrosa, Regina Palmeiro Carballeira, Ricardo V. García-Mayor

Servicio de Endocrinología y Nutrición, Complejo Hospitalario Universitario de Vigo, Hospital Xeral, Vigo, Pontevedra, Spain
Servicio de Cirugía General y Digestiva, Complejo Hospitalario Universitario de Vigo, Hospital Xeral, Vigo, Pontevedra, Spain
Servicio de Anatomía Patológica, Complejo Hospitalario Universitario de Vigo, Hospital Xeral, Vigo, Pontevedra, Spain

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Abstract

Background and objectives: Genetic testing of the RET proto-oncogene allows for early diagnosis of multiple endocrine neoplasia syndrome type 2 and establishes a correlation between genotype and clinical manifestations. The purpose of this study was to demonstrate the benefits of early diagnosis with genetic testing followed by prompt surgery for curing medullary thyroid carcinoma (MTC) as compared to later diagnosis with serum calcitonin.

Patients and method: A retrospective, descriptive study of 8 members of a family with MEN 2A due to C634Y mutation. We performed serum calcitonin screening until 1999, and subsequently RET genetic testing. The carriers underwent total thyroidectomy, periodic determination of calcitonin, urinary metanephrines, calcium, and phosphorus, and cervical and abdominal imaging techniques.

Results: Five patients were diagnosed by calcitonin familial screening and at the time of writing all of them had high calcitonin levels. Three patients were diagnosed by genetic testing (an adult and two children) and were free of disease. Calcitonin was closely monitored in the children, who underwent surgery when it started to rise at 6 and 10 years of age respectively, nodular C-cell hyperplasia having been found in both. Three of the eight carriers developed bilateral and asynchronous pheochromocytoma, half had normal urinary metanephrine levels and two also had MTC. No patient had biochemical data suggesting hyperparathyroidism although in one patient multiple parathyroid adenomas were found at thyroidectomy.
Introduction

Multiple endocrine neoplasia type 2 (MEN2A) syndrome was described in 1961 by Sipple, who proposed a connection between bilateral pheochromocytoma and thyroid carcinoma. Steiner subsequently coined the term MEN2 and noted that the phenotype was wider and included medullary thyroid carcinoma (MTC), pheochromocytoma, ganglioneuroma, and Marfanoid habit. Familial MTC (FMTC) is an incomplete variant in which only MTC occurs, but is occasionally associated with Hirschsprung’s disease or cutaneous lichen amyloidosis.

MEN2 is a genetic disorder of an autosomal dominant inheritance with an almost 100% penetrance caused by missense mutations (substitution of an amino acid for another) in the germ line of the RET proto-oncogene (rearranged during transfection). The RET gene is located in chromosome 10 (10q11.2) and consists of 21 exons. The gene encodes for a tyrosine kinase receptor which is expressed in cells derived from the neural crest and is involved in cell growth and differentiation processes. Its mutations mainly affect four types of tissue, all of them derived from the neural crest: thyroid C cells, parathyroid cells, chromaffin cells from adrenal medulla and enteric autonomic plexus. In MTC, tumor development occurs as a result of constitutive receptor activation, which in MEN2A leads to accelerated cell proliferation. Each tissue has a different sensitivity to RET activation, and pheochromocytoma and hyperparathyroidism therefore only occur in certain mutations, especially those in codon 634.

RET mutation is thought to be in the ratio of 1 carrier per 500,000 inhabitants/year. The estimated prevalence of
MEN2 is 1/30,000 inhabitants, and MEN2A is found in more than 80% of cases\textsuperscript{8}. Age at MTC presentation and frequency of parathyroid and adrenal medulla involvement do not usually depend on the type of amino acid replacing the original one, but on the codon where this replacement occurs, although some specific mutations are related to the course of the disease\textsuperscript{8}. It has been postulated that the risk of progression depends on the transformation potential of the mutation in each individual\textsuperscript{8}. Different RET mutations associated with MEN2A have been reported to date, of which mutations in codon 634 are the most common, accounting for 80% of germline RET mutations. This mutation results in hyperparathyroidism while others do not\textsuperscript{14}, but environmental factors, such as obesity, can influence the onset of hyperparathyroidism. Mutation in codon 634 is closely related to pheochromocytoma\textsuperscript{4,11} and sporadically related to hyperparathyroidism. Mutation in codon 634 is associated with cutaneous lichen amyloidosis and is not causally related to Hirschsprung’s disease.

In this regard, a genotype-phenotype correlation is known to exist, i.e. if the mutated codon is known, the age at which MTC will develop may be predicted. This has led to groups at risk being identified and specific recommendations being made regarding both the timing and type of surgery based on the youngest patient diagnosed with MTC, the youngest age at which nodal metastases occur, and the mean age of MTC onset in each family with a given mutation\textsuperscript{14}. Prophylactic thyroidec­tomy in mutation carriers has changed the natural history of the disease, and is the most representative example of primary prevention of a genetic cancer\textsuperscript{13}.

It is not exactly known why some members of a family with the same mutation develop pheochromocytoma or hyperparathyroidism while others do not\textsuperscript{14}, but environmental or genetic factors such as RET polymorphisms have been proposed. Such factors, together with the occurrence of somatic mutations, could account for the genetic anticipation phenomenon by which the phenotype becomes more aggressive with each successive generation. Hence the importance of lifetime monitoring of people with RET mutations.

A descriptive, evolutionary study was conducted in a family with MEN2A due to a C634Y mutation in RET proto-oncogene followed up for more than 20 years.

Subjects and method

Twenty people belonging to three generations of a family with MEN2A due to a C634Y mutation were studied. First-degree relatives of the index case (patient 1, Table 1), diagnosed with MTC in 1989, were studied by serum calcitonin measurements up to 1999, and then with genetic testing of the RET proto-oncogene, which was also performed in the children of carriers. One side of the family refused follow-up (Fig. 1). Mutation could have been transmitted through the paternal line, as the mother of the index case died at 87 years, while the father died before 40 years of age from an unknown cause.

MTC monitoring consisted of regular measurement of serum calcitonin levels, neck ultrasound, and an octreoscan from the year 2000 when increased serum calcitonin levels were found and no residual thyroid tissue was shown by imaging techniques (neck ultrasound and neck and chest CT scan).

All carriers underwent annual urinary metanephrine tests by HPLC and abdominal imaging techniques: MRI every three years in adults and annual ultrasonography in children. Calcium and phosphorus levels were annually monitored to detect hyperparathyroidism, but have been normal to date in all patients.

Results

Medullary thyroid carcinoma

Of the 8 patients, 3 were diagnosed by genetic testing, two of them at 2 and 3 years of age (patients 7 and 8) and one at an adult age (patient 5). In children, annual calcitonin measurements were performed, and elective surgery was decided when calcitonin levels reached the upper limit of normal, which occurred at 10 years in patient 7 and at 6 years in patient 8. Surgery consisted of total thyroidectomy, and pathological examination revealed nodular C-cell hyperplasia in both patients. Seven years later, they were free of disease and had no sequelae derived from surgery. Patient 5 was diagnosed at 34 years of age and underwent total thyroidectomy with central lymphadenectomy. Multicentric MTC with no nodal involvement was detected, and at the time of writing the patient had no evidence of disease either.

As the high calcitonin levels would suggest, the index case and the 4 relatives diagnosed by calcitonin screening were not cured, although long-term remissions were seen in some of them (Table 1). Cervical lymphadenectomy was not performed in any of them, and we therefore do not know whether there were lymph node metastases at diagnosis.

The mean duration of disease remission, defined as undetectable or normal baseline serum calcitonin levels, was 11.8 years.

Pathological examination revealed different varieties of MTC, which was multicentric and bilateral in all patients, and in patient 5 was also associated with parathyroid hyperplasia.

Pheochromocytoma

Three of the 8 patients had bilateral and asynchronous pheochromocytoma. Three were detected by high urinary metanephrine levels, and three by imaging techniques. The index case (patient 1) was the only patient who underwent surgery without a prior study of pheochromocytoma. Urinary metanephrine levels were normal in patient 5 before thyroid surgery, but subsequent imaging techniques showed the presence of an adrenal mass which turned out to be a pheochromocytoma.

Hyperparathyroidism

Only patient 5 experienced parathyroid hyperplasia, which was not diagnosed before thyroid surgery because he had normal calcium and phosphorus levels. Resection
characterization has resulted in a total change in management thanks to early diagnosis and treatment, which has had a satisfactory impact on its prognosis. Genetic testing has allowed not only for early diagnosis of MTC, but also for carrier management before disease development, unlike lifetime monitoring of serum calcitonin levels in all first-degree relatives. In our study, elevated calcitonin levels already reflected the presence of tumor. Early treatment for MTC represents the paradigm for the primary prevention of hereditary cancer in humans13. On the other hand, the presence of a given mutation is associated with a characteristic clinical presentation, which allows for a genotype-phenotype correlation which is essential for patient management.

Table 1  Characteristics of medullary thyroid carcinoma in a MEN2A family

<table>
<thead>
<tr>
<th>Patient</th>
<th>Form of diagnosis</th>
<th>Age at S</th>
<th>Year of S</th>
<th>Type of S</th>
<th>Pathology</th>
<th>Free of disease</th>
<th>Time free of disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Index case. Histological study</td>
<td>48</td>
<td>1989</td>
<td>Near-total thyroidectomy</td>
<td>Multicentric MTC</td>
<td>No</td>
<td>17 years</td>
</tr>
<tr>
<td>2</td>
<td>Calcitonin screening</td>
<td>58</td>
<td>1995</td>
<td>Extended total thyroidectomy</td>
<td>Intrathyroid bilateral MTC</td>
<td>No</td>
<td>9 years</td>
</tr>
<tr>
<td>3</td>
<td>Calcitonin screening</td>
<td>31</td>
<td>1990</td>
<td>Total thyroidectomy</td>
<td>MTC throughout the specimen</td>
<td>No</td>
<td>6 years</td>
</tr>
<tr>
<td>4</td>
<td>Calcitonin screening</td>
<td>28</td>
<td>1990</td>
<td>Total thyroidectomy</td>
<td>MTC with follicular foci</td>
<td>No</td>
<td>16 years</td>
</tr>
<tr>
<td>5</td>
<td>Genetic study</td>
<td>34</td>
<td>2000</td>
<td>Total thyroidectomy and central lymphadenectomy</td>
<td>Multicentric MTC. Parathyroid hyperplasia</td>
<td>Yes</td>
<td>9 years</td>
</tr>
<tr>
<td>6</td>
<td>Calcitonin screening</td>
<td>23</td>
<td>1991</td>
<td>Total thyroidectomy</td>
<td>Multifocal MTC</td>
<td>No</td>
<td>11 years</td>
</tr>
<tr>
<td>7</td>
<td>Genetic study</td>
<td>10</td>
<td>2002</td>
<td>Total thyroidectomy</td>
<td>Nodular C-cell hyperplasia</td>
<td>Yes</td>
<td>7 years</td>
</tr>
<tr>
<td>8</td>
<td>Genetic study</td>
<td>6</td>
<td>2002</td>
<td>Total thyroidectomy</td>
<td>Nodular C-cell hyperplasia</td>
<td>Yes</td>
<td>7 years</td>
</tr>
</tbody>
</table>

S: surgery.

of grossly enlarged parathyroid glands caused severe hypoparathyroidism with high calcium and vitamin D requirements.

Other

As regards other findings associated with MEN2, cutaneous lichen amyloidosis was found in two patients. None of them had Hirschsprung’s disease. Table 2 shows other findings unrelated to MEN2.

Discussion

MEN2A or syndrome of multiple endocrine neoplasia type 2A is due to mutations in the RET proto-oncogene. Its characterization has resulted in a total change in management thanks to early diagnosis and treatment, which has had a satisfactory impact on its prognosis. Genetic testing has allowed not only for early diagnosis of MTC, but also for carrier management before disease development, unlike lifetime monitoring of serum calcitonin levels in all first-degree relatives. In our study, elevated calcitonin levels already reflected the presence of tumor. Early treatment for MTC represents the paradigm for the primary prevention of hereditary cancer in humans13. On the other hand, the presence of a given mutation is associated with a characteristic clinical presentation, which allows for a genotype-phenotype correlation which is essential for patient management.

Mutation in codon 634 is the most common and, obviously, the best known of the multiple mutations reported. This
Phenotype of the C634Y mutation in the RET proto-oncogene in MEN2A: report of a family

The C634Y mutation causes an impairment in the tyrosine kinase receptor encoded by RET, conferring on it a gain of function by ligand independent receptor dimerization and constitutive activation. This mutation has been associated with all signs reported in MEN2A.

In our series, only patients treated early due to the genetic study were free of disease, while all the others showed evidence of persistence or recurrence. The poorer course in the biochemically diagnosed group could be attributed to the lack of the prophylactic central lymphadenectomy recommended by current guidelines, or to the fact that a calcitonin-based diagnosis, made at a later time, could have been associated with a poorer prognosis because the condition was at a more advanced stage. This was not histologically confirmed because lymphadenectomy and biopsy of lateral cervical and mediastinal lymph nodes were not performed, as these patients underwent surgery years before the current guidelines recommending these practices were issued.

Medullary thyroid carcinoma

MTC in mutation 634 occurs early, is aggressive, and has a great metastasizing capacity, which involves high persistence or recurrent rates after surgery. Disease-free intervals up to 17 years followed by recurrence have been found in our patients. MTC development starts with C-cell hyperplasia, a pretumoral stage, which evolves very differently over time, forming bilateral and multicentric foci. This is the first neoplasm to develop and is the most common cause of death in patients with MEN2. No deaths occurred in our series, despite the fact that 10-year survival rates ranging from 71%-100% have been reported in the literature for patients with stage I-III MTC. The rate decreases to 21% in stage IV MTC, but there are no specific data for MEN2A.

Prophylactic thyroidectomy is the usual practice in disease carriers. While surgery could be indicated before the age of 5 years because this is a high-risk mutation, we decided to perform close monitoring and to program surgery if slightly elevated calcitonin levels, even within the normal range, were found. This decision was agreed with the parents because they were reluctant to surgery in infancy. It should be noted that the age of presentation in this family had not been particularly young and its course could be considered benign, with a 100% survival rate to date. While family members were aware of the genetic study, they had complete confidence in the diagnostic value of calcitonin. Prophylactic thyroidectomy was therefore performed at 6 and 10 years of age in patients 7 and 8, and pathological examination revealed C-cell hyperplasia.

Genotype-phenotype correlation has some limitations. Mutations in certain codons are known to be able to cause different clinical expressions, while those in exons 10 and 11 (extracellular domain) are associated with MEN2A or FMTC. Some mutations are associated with the development of MTC and other endocrine tumors when they occur in homozygosis or are associated with somatic mutations. Age at MTC occurrence may vary if the normal RET allele is lost or the mutated allele is duplicated. In addition, although the genotype-phenotype correlation is useful for MTC, it does not accurately predict for development of hyperparathyroidism or pheochromocytoma or the age at which they will occur.

Pheochromocytoma

Pheochromocytoma in MEN2A is more common with the 634 mutation than with other mutations. It is typically bilateral, does not experience malignant transformation, and is not found in extra-adrenal locations. Pheochromocytoma is the first sign of MEN in 25% of cases, is concomitant with MTC in 35%, and occurs after the latter in 40%-50% of cases. In our series, pheochromocytoma was not diagnosed before MTC in any patient. Mutations in codon 634 have been found in children aged 5 to 10 years, and screening should therefore be started before thyroidectomy or any other surgery is performed and repeated every year. The measurement of

<table>
<thead>
<tr>
<th>Patient</th>
<th>Pheochromocytoma</th>
<th>High metanephrine levels</th>
<th>Synchronous with MTC</th>
<th>Hyperparathyroidism</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bilateral</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Café-au-lait spots</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td></td>
<td></td>
<td>No, postoperative</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>hypoparathyroidism</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Cutaneous lichen</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No</td>
<td>amyloidosis</td>
</tr>
<tr>
<td>3</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Café-au-lait spots</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No</td>
<td>Wolf-Parkinson-White</td>
</tr>
<tr>
<td>4</td>
<td>No</td>
<td>No</td>
<td>Cafe-au-lait spots</td>
<td>Yes</td>
<td>Cutaneous lichen</td>
</tr>
<tr>
<td>5</td>
<td>Bilateral</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>amyloidosis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Hepatic carcinoid</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Bilateral</td>
<td>No (1st)</td>
<td>Yes</td>
<td>No</td>
<td>Tumor</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Si (2nd)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Tumors associated with MEN2 and other clinical findings
fractionated catecholamines and metanephrines in 24-hour urine is advised for screening. Some recommend the testing of serum metanephrines because of their greater sensitivity, but this is not supported by some studies. In our series, only half the patients had high urinary metanephrine levels. Guidelines advise the performance of imaging tests at the time of biochemical diagnosis and every 3 to 5 years after surgery required by permitting phenotype-genotype correlation. It also guided us as to both the type of and the timing of the surgery required for maintaining remission after surgery in already developed MTCs.

**Conflict of interest**

The authors state that they have no conflict of interest.

**References**


**Table 3** Risk group stratification according to the 7th International MEN Meeting 1999

<table>
<thead>
<tr>
<th>Risk group</th>
<th>Indication of surgery time and type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1: low risk. Codons 609, 768, 790, 791, 804, 891</td>
<td>Total thyroidectomy at 5-10 years. Or annual calcitonin testing and elective surgery when high calcitonin levels are found</td>
</tr>
<tr>
<td>Group 2: high risk. Codons 611, 618, 620, 634 lymphadenectomy before 5 years of age</td>
<td>Total thyroidectomy with or without central</td>
</tr>
<tr>
<td>Group 3: maximum risk. MEN2B. Codons 883, 918, and 922</td>
<td>Total thyroidectomy with central lymphadenectomy in the first 6 months of life</td>
</tr>
</tbody>
</table>

**Hyperparathyroidism**

Hyperparathyroidism is the least common finding in MEN2A. It is associated with mutations in codon 634, and annual measurement of serum calcium and PTH is therefore recommended because it is most often asymptomatic. In a normocalcemic patient with MEN2A, one or more enlarged parathyroid glands may be found, as occurred in patient 5. Prophylactic resection may lead to permanent hypocalcemia and is questionable.

**Lichen amyloidosis**

This is a pruritic skin lesion, usually located in the upper back. Not all patients with lichen amyloidosis have RET mutations, but when the condition is associated with MTC there are usually mutations in codon 634, except for the case of one patient with mutation in codon 804. Lichen amyloidosis is thought to be possibly related to a sensory abnormality in dermatomes C6-T6, which would lead to neurological pruritus and amyloid deposit as a consequence of repeated scratching. Two cases were seen in our family, both detected several years after MTC diagnosis, although the condition has also been reported before MTC.

**Conclusions**

MEN2A is a genetic syndrome with varied clinical signs and symptoms causing significant morbidity. Genetic study of the RET proto-oncogene allowed for early diagnosis and treatment of MTC in our patients, and has been determinant for maintaining remission after surgery in already developed MTCs. It also guided us as to both the type of and the timing of the surgery required by permitting phenotype-genotype correlation. Mutation 634 is an example of how a genetic change is associated with a disease that may be predicted and managed, thus significantly improving prognosis in these patients.
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