CLINICAL STUDY

Multiple endocrine neoplasia type 2A in two families with the familial medullary thyroid carcinoma associated G533C mutation of the RET proto-oncogene

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Abstract

Introduction: Multiple endocrine neoplasia type 2A (MEN2A) is an autosomal dominant hereditary disorder, associated with a cluster of germline gain-of-function mutations of the RET proto-oncogene (RET), mainly in exons 10–15. The G533C mutation in exon 8 of the RET is rare and has been mainly related to the familial medullary thyroid carcinoma. Patients-methods: We describe the RET G533C mutation in exon 8 of the RET in two unrelated female index patients, with MEN2A phenotype, consisting of pheochromocytoma which was the presenting feature and medullary thyroid carcinoma. In addition, 12 family members were also studied. DNA extraction, PCR, and sequencing of RET was performed in exons 7–19 and 21, following standard procedures. Results: The mutation was found in both index patients and in 6 out of 12 family members (50%). Three of them were biochemically affected with histologically proven medullary thyroid carcinoma in two of them while there are no certain clues regarding the other three members as they declined further evaluation. Conclusion: Patients with MEN2A should be also searched in exon 8 while positive carriers of this mutation should be screened annually for pheochromocytoma or other components of the syndrome.

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Introduction

Multiple endocrine neoplasia type 2 (MEN2) is an autosomal dominant disorder which is characterized by the combined occurrence of endocrine tumors and abnormalities in nonendocrine tissues (1). MEN2 is caused by germline gain-of-function mutations of the RET proto-oncogene (RET) (2). Mutations affecting the cysteine-rich extracellular domain encoded in RET exons 10–15 have been associated with MEN2A; a single point mutation in RET exons 15 and 16 has been associated with MEN2B; mutations at certain codons in various exons of RET (5, 8, 11–16) have been associated with familial medullary thyroid carcinoma (FMTC) (1, 3–7). However, the G533C mutation in exon 8 of the RET is a rare mutation and has been mainly associated with FMTC (8).

In this paper, we describe the G533C mutation in exon 8 of the RET in two unrelated female Greek patients with MEN2A phenotype consisting of pheochromocytoma (PHEO) which was the presenting feature and medullary thyroid carcinoma (MTC). In addition, we present data regarding the clinical characteristics, the biochemical profile, and the genetic analysis of 12 family members from both index patients who were involved in the study.

Family A

Index patient A was a 35-year-old woman complaining of hypertensive episodes accompanied with palpitations, headache, and sweating. During her evaluation, she had an abdominal CT-scan which revealed a left adrenal mass (diameter 5.8×4.5 cm). Repeated screening for the diagnosis of PHEO was negative (normal urinary vanillylmandelic acid (VMA), metanephrines, and catecholamines). She underwent laparoscopic left adrenalectomy, during which she suffered a severe complicated hypertensive crisis and needed to be hospitalized in intensive care unit. Histology report showed an adrenal PHEO that was stained positive for chromogranin, inhibin A, and S100 protein with a low Ki67 index (less than 2%). The young age of PHEO presentation raised the suspicion of a hereditary syndrome and further work-up was suggested during which high-normal calcitonin (CT)
values (14.6, \text{nv} < 13 \text{ ng/l}) and a positive pentagastrin (Pg) stimulation test \text{(max calcitonin (CT) value 309 ng/l)} were found (9). The patient underwent total thyroidectomy with central compartment node dissection and the histology showed multifocal medullary carcinoma and C-cell hyperplasia (CCH) while a microscopic papillary thyroid carcinoma (PTC) was also found.

A total of eight relatives from two generations were also studied. The father was not clinically affected, he had elevated basal CT levels (14 ng/l, \text{nv} < 13) but he refused any further biochemical evaluation. Regarding her three sisters, two of them had hypertension starting from a young age while the biochemical evaluation for PHEO and MTC was negative. Among her four brothers, two had elevated basal and post Pg stimulation CT levels (mean basal: 23.5 ng/l, \text{nv} < 13, mean stimulated: > 100 ng/l), normal biochemical testing for PHEO, and negative abdominal CT imaging (Table 1). Based on those results, they underwent total thyroidectomy and the histology confirmed the diagnosis of MTC (Table 1, Fig. 1).

Family B

Index patient B was a 48-year-old woman with a left adrenal incidentaloma (diameter 6 × 5.5 cm). Further investigation showed elevated levels of urinary VMA (67.1 \text{nv} < 32.8 \text{ μmol/d}) and norepinephrine (626.4 \text{nv} < 591 \text{ nmol/d}) and a positive metaiodobenzylguanidine (MIBG) scan. After proper treatment with phenoxybenzamine, she underwent left adrenalectomy and the histology showed PHEO which was stained positive for chromogranin (Fig. 2a). The relatively young age of PHEO presentation raised the suspicion of a genetic syndrome and the following evaluation showed elevated basal CT levels (mean basal: 60.6, \text{nv} < 13 ng/l). The patient underwent total thyroidectomy and the histology showed MTC, stained positive for CT, monoclonal carcinoembryonic antigen (CEA), chromogranin, synaptophysin, and CD57/Leu 7 (Fig. 2 b and c). A total of four relatives from two generations were also studied. Her father did not have any evident clinical signs, he had normal CT levels but he did not complete the suggested biochemical testing. Her two sons and her mother were not clinically affected while they did not complete the suggested biochemical evaluation (Table 1, Fig. 1).

Methods

Urinary VMA and catecholamines were determined by HPLC (LaChrom D7000, Merck-Hitachi). Serum CT levels were determined by a chemiluminescence CT immunoassay (Siemens Medical Solutions Diagnostics Ltd, Glyn Rhonwy, Llanberis, Caernarfon, UK).

The Pg stimulation test consisted of a pulse administration of 0.5 μg/kg Pg (Pg Injection BP, Cambridge Laboratories, Tyne & Wear, UK). Blood was collected before and at 2, 5, and 10 min after the injection. CT values were considered normal with reference to the data provided (normal values < 13 ng/l).

Genetic analysis

Informed consent was obtained from all individuals and genomic DNA was prepared from peripheral blood samples collected on EDTA tubes according to standard protocols. Sequencing was performed in an ABI3100 genetic analyzer. The genetic protocol included the screening for \textit{RET} mutations in exons 7–19 and 21, according to standard procedures (10–14).

Results

Direct sequencing of exon 8 revealed a G to T transversion at position 1597, in both index patients with MEN2A phenotype consisting of PHEO as the presenting feature and MTC. This results in the substitution of glycine to cysteine residue at codon 533 (Gly533Cys) in the cysteine-rich extracellular domain of the \textit{RET} protein. In addition, two common polymorphisms G691S and S904S were detected in index patient A.

Table 1  Clinical characteristics and radiological data of the mutation-positive kindred members.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>Preoperative basal CT/Pg-CT (pg/ml)</th>
<th>Thyroid imaging</th>
<th>Thyroid histology</th>
<th>Lymph node metastases</th>
<th>Adrenal imaging</th>
<th>PHEO</th>
<th>HPT</th>
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<tr>
<td>Family A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>I-1</td>
<td>M</td>
<td>67</td>
<td>14/NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>II-2</td>
<td>F</td>
<td>34</td>
<td>8.9/309</td>
<td>Negative</td>
<td>MTC/CCH</td>
<td>No</td>
<td>LA mass</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>II-3</td>
<td>M</td>
<td>33</td>
<td>2.2/23</td>
<td>Negative</td>
<td>NA</td>
<td>NA</td>
<td>Negative</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
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<td>M</td>
<td>27</td>
<td>34/74</td>
<td>ND</td>
<td>MTC</td>
<td>No</td>
<td>Negative</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>II-6</td>
<td>M</td>
<td>26</td>
<td>13.1/102</td>
<td>Negative</td>
<td>MTC/CCH</td>
<td>No</td>
<td>Negative</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Family B</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>I-1</td>
<td>M</td>
<td>86</td>
<td>2.9/NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>II-1</td>
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<td>48</td>
<td>74/NA</td>
<td>ND</td>
<td>MTC/CCH/PTC</td>
<td>No</td>
<td>LA mass</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>III-2</td>
<td>M</td>
<td>24</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

M, male; F, female; CT, calcitonin; Pg, pentagastrin; PHEO, pheochromocytoma; HPT, hyperparathyroidism; MTC, medullary thyroid carcinoma; CCH, C-cell hyperplasia; PTC, papillary thyroid carcinoma; ND, nodular goiter; LA, left adrenal and NA, not available.
Genetic screening of family A identified four positive gene carriers (the father and three brothers) while her three sisters were found to be negative. Among the brothers, two out of the three had histologically confirmed MTC (Table 1) while the third brother was a positive carrier with no clinical and/or biochemical signs of the disease, at present. The clinically asymptomatic father was a positive carrier, with elevated basal CT levels with no certain diagnostic clues as he declined further evaluation.

Genetic screening of family B revealed two positive gene carriers (the father and one son) who were not clinically affected without certain clues as they did not complete their biochemical evaluation. The mother and the other son were negative for the mutation (Fig. 2).

In total, 6 out of 12 family members (50%) were found to be positive for the mutation. Three of them (50%) were biochemically affected with histologically proven MTC in two (33%) of them, while in three (50%) there are no certain diagnostic clues as they did not complete the suggested evaluation.

Discussion

This is a well-documented report of a G533C mutation in exon 8 of the RET in two Greek unrelated index patients with MEN2A phenotype consisting of PHEO which was the presenting feature and MTC. Fifty percent of the studied family members were positive carriers, 50% of them had MTC based on the biochemical results and the histology report with no other clinical and/or laboratory signs of the syndrome, at this time.

According to the international RET mutation consortium analysis (1), a strong genotype/phenotype correlation has been found between the MEN2 phenotype and the various mutations of the RET gene in contrast to the other known hereditary multiple endocrine neoplasia syndrome, MEN1 (10, 15). Mutations related to MEN2A concern mainly the cysteine-rich extracellular domain of the RET encoded in exons 11 (codon 634, 630, 635, 637, and 666) and 10 (codons 609, 611, 618, and 620), leading to RET dimerization and constitutive activation of downstream signaling pathways (1, 3–7). Less often, MEN2A-related mutations concern the exon 13 (codons 768, 790, and 791), 14 (codon 804), and 15 (codon 891) (1, 3). However, mutations of the RET gene, regarding exon 8, are quite rare and have been mainly associated with FMTC. The first report by Pigny et al. (11) described a 9 bp duplication of exon 8 in a family with FMTC. Furthermore, Da Silva et al. (12) and Kaldrymides et al. (8) described the G533C point mutation in exon 8 of the RET gene in three large families consisted of 96 subjects with FMTC phenotype. Our study supports that the G533C point mutation in exon 8 of the RET gene is possibly associated with MEN2A phenotype. To our knowledge, there is only one case report of a 66-year old patient with MEN2A harboring the reported mutation (16) (Table 2). Obviously the major limitation

<table>
<thead>
<tr>
<th>Author (Ref)</th>
<th>Kindreds</th>
<th>Number of mutation-positive patients</th>
<th>Phenotypic expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Da Silva et al. (14)</td>
<td>1</td>
<td>76</td>
<td>FMTC</td>
</tr>
<tr>
<td>Kaldrymides et al. (10)</td>
<td>2</td>
<td>20</td>
<td>FMTC</td>
</tr>
<tr>
<td>Bethanis et al. (11)</td>
<td>1</td>
<td>1</td>
<td>MEN2A</td>
</tr>
</tbody>
</table>

FMTC, familial medullary thyroid carcinoma and MEN2A, multiple endocrine neoplasia type 2A.
of that report was the fact that it included only one patient. Thus, in the absence of genetic testing in other family members, no facts as to the mutation penetrance or the phenotypic expression in younger carriers were available.

It has to be mentioned that in our study as in the report by Bethanis et al. (16), PHEO was the presenting feature while most commonly MTC is diagnosed earlier in the course of the syndrome (1, 10). Additionally, one of our index patients was asymptomatic, the PHEO being accidentally found, while the second patient had hypertension but negative testing for PHEO despite repeated measurements. Furthermore, the MTC was least aggressive as it was not clinically apparent, while none of the family members died from MTC-related causes. The above observations are considered quite interesting points in the characterization of the MEN2A phenotype associated with the G533C point mutation in exon 8 of the RET gene which seems to be less aggressive. However, the possibility of the association of the G533C mutation with FMTC while the PHEO could be sporadic cannot fully be ruled out at present. However, additional studies in Greek families are in progress and eventually this issue will be resolved in the near future (17). The further follow-up of those patients as well as of an extended study population of MEN2A affected individuals concerning this transition in exon 8 will give insight into the exact way of penetrance and the natural history of the MEN2A associated with this mutation.

In addition to the G533C mutation, two common polymorphisms G691S and S904S at exons 11 and 15 respectively were found in the index patient A. Literature data suggest that these single nucleotide polymorphisms may possibly have a modulatory effect on the clinical expression of MEN2 in addition to the sporadic MTC (18–20).

Furthermore, papillary microcarcinoma was found in addition to MTC in index patient A. This rare concurrence, named collision tumor, has been described in a number of patients with MEN2. This is a quite interesting finding as it combines two pathologic conditions with a different genetic background. According to the existing knowledge, the PTC harbors a BRAF somatic mutation and not a RET/PTC rearrangement. However, a common genetic drive for MTC and PTC cannot be excluded (21).

This study in accordance with other existing studies represents one of the best examples, where molecular biology changed the way of evaluation and further therapeutic intervention of a hereditary cancer syndrome such as MEN2 (22, 23).

In conclusion, the G533C point mutation of the RET gene seems to be related to MEN2A and possibly to a milder phenotype of the syndrome. Moreover, patients with MEN2A should also be searched in exon 8 while positive carriers should be screened annually for PHEO or other components of the syndrome.

Declaration of interest

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

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References


