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A Korean Family of Familial Medullary Thyroid Cancer with Cys618Ser RET Germline Mutation

Familial medullary thyroid carcinoma (FMTC) is caused by autosomal dominant gain-of-function mutations in the RET proto-oncogene. An identifiable RET mutation can be detected in about 85% of FMTC families. The majority of germline mutations in FMTC have been found in exons 10 and 11 of the RET proto-oncogene, specifically within the cysteine codons 609, 611, 618, 620, and 634. We screened members of a large Korean family that had a history of FMTC by genetic analyses, and propose a therapeutic approach for managing the disorder. We report a RET mutation in exon10, codon 618 that causes substitution of a cysteine by a serine in the cysteine-rich domain of the RET receptor in a three-generation FMTC family composed of 30 members with 11 carriers. Nine of the gene carriers were clinically affected. The FMTC with cysteine RET mutations found in the Korean population is consistent with the clinical pattern reported worldwide; to date there have been no ethnic differences identified for FMTC. Our results suggest that this genetic profile might be associated with usually aggressive clinical course with regional lymph node metastasis but late onset of MTC.

Key Words : *Familial Medullary Thyroid Carcinoma; RET Mutation; Genetic Testing*

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INTRODUCTION

Medullary thyroid carcinoma (MTC) represents 3% to 10% of most institutional series of thyroid cancers; hereditary MTC accounts for 25% to 30% of these patients (1, 2). Hereditary MTC is divided into three clinical subtypes depending on the presence or absence of tissue-specific tumors, phenotypic characteristics and the number of affected family members: familial MTC (FMTC), multiple endocrine neoplasia (MEN) type 2A and MEN type 2B (3, 4). FMTC accounts for 5% to 15% of hereditary MTC cases. It is defined as the presence of MTC in a kindred with four or more affected members, and with no clinical or biochemical evidence of adrenal or parathyroid gland involvement either in the affected members or in the available first degree relatives (5). FMTC represents a less aggressive form of hereditary MTC, and it has a corresponding older age at onset, often between 20 and 40, compared to MEN 2A and MEN 2B (6, 7).

Hereditary MTC is caused by autosomal dominant gain-of-function mutations in the RET proto-oncogene (8). A germline mutation in the RET proto-oncogene, which encodes for a transmembrane tyrosine kinase receptor, predisposes individuals to develop MTC. An identifiable RET mutation can be detected in about 85% of FMTC families (9). The majority of germline mutations in FMTC have been found in exons 10 and 11 of the RET proto-oncogene, specifically within the cysteine codons 609, 611, 618, 620, and 634. In addition, mutations in the intracellular domain of the protein have been described at: 768, 790, 791 in exon 13, V804M, 844 in exon 14, 891 in exon 15, and 912 exon 16 (3, 7, 10). Mutations at codons 532, 533 (exon 8), 768, 844, and 912 have been identified only in families with FMTC (10-12).

Many studies of RET mutations in heritable MTC have been published in different countries. However, there are few reports of these mutations in Korean families with FMTC. In this study we screened members of a large Korean family that had a history of FMTC by genetic analyses, and propose a therapeutic approach for managing the disorder.

MATERIALS AND METHODS

The pedigree of the family is shown in Fig. 1. This large family consists of three generations with a total of 30 individuals. Twenty-nine members of the family underwent genetic testing in 2004. There was a history of MTC in eight mem-

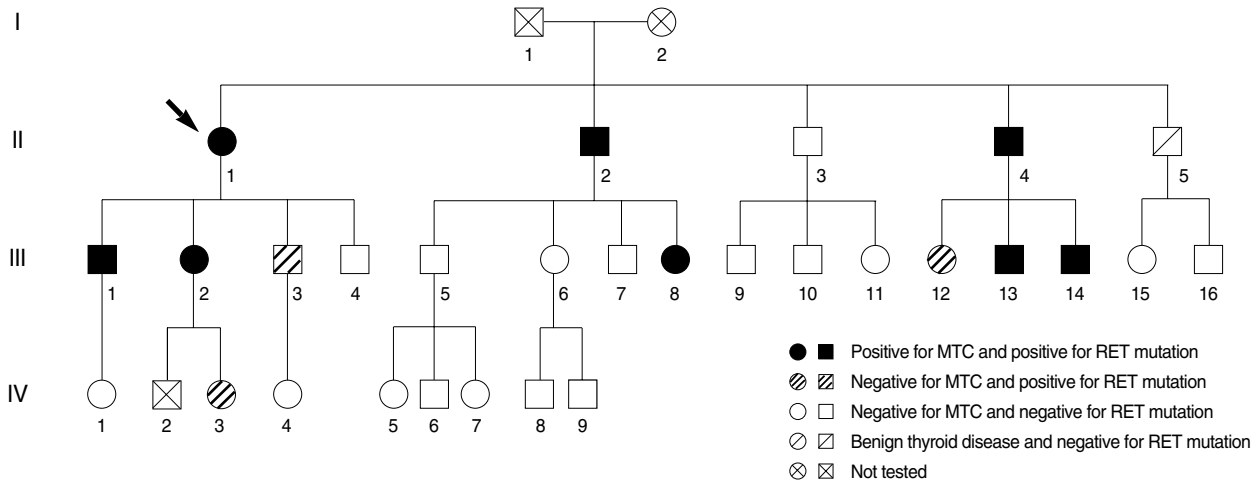

Fig. 1. Pedigree of the family with familial medullary thyroid carcinoma. Circles and squares denote female and male family members, respectively.

bers of the family. Five patients were diagnosed with MTC before genetic testing (II-1, II-4, III-2, III-8, III-14). Other three patients were diagnosed after genetic testing and clinical screening (II-2, III-1, III-13). The index case was a 67-yr-old woman (II-1) who had a total thyroidectomy with modified radical neck dissection bilaterally, at the age of 48 yr, for an anterior neck mass that presented 10 yr earlier. The histological examination showed the presence of MTC in both lobes and microscopic metastatic disease in 14 lymph nodes in the central and both lateral compartments. There was no clinical or biochemical evidence of pheochromocytoma, parathyroid disease or other associated clinical features attributed to MEN2. On her last follow-up visit, 23 yr after her initial diagnosis, there was no radiological evidence of recurrent or metastatic disease, despite an elevated serum calcitonin level of 593.5 pg/mL (normal range, <10 pg/mL). The index patient and at-risk family members had RET mutation genetic screening. Prior to obtaining the samples, all individuals included in the study were fully informed about the study and consents were obtained. After the genetic screening, we recommended further evaluation for gene carriers. However, they refused further biochemical testing and agreed only to thyroid ultrasound examinations.

DNA analysis

For identification of RET germline mutations, genomic DNA was purified from peripheral blood lymphocytes using the QIA quick PCR purification kit (Qiagen, Hiden, Germany). DNA of the index case (II-1) was first screened by looking for RET proto-oncogene mutations in exons 10, 11, 13, 14, 15, and 16, where most of the RET mutations are found in FMTC. For the rest of the individuals, only exon 10 was analyzed. Genomic DNA was amplified by the polymerase chain reaction with oligonucleotide primers for exons

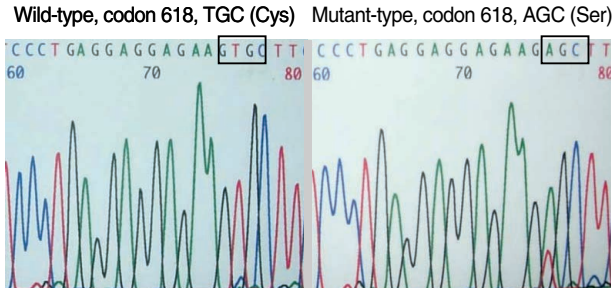

Fig. 2. The sequence TGC encoding cysteine in the wild-type is changed to the sequence AGC encoding serine in codon 618 on exon 10 of the RET proto-oncogene.

10, 11, 13, 14, 15, and 16. Single-strand conformation polymorphism analysis and direct sequencing were performed on an automated DNA sequencer (ABI PRISM 310, Perkin Elmer, Foster City, CA, USA).

RESULTS

Genetic analysis of the index case revealed a missense TGC →AGC mutation at codon 618 in exon 10 of the RET proto-oncogene. This transversion leads to the substitution of cysteine with serine (Fig. 2). Direct sequencing of the RET exon 10 polymerase chain reaction products, from genomic DNA of family members, showed that 11 of 29 members were carriers for this mutation, and 18 were unaffected. Clinical features of the mutation of the carriers are shown in Table 1. Nine of the gene carriers were clinically affected. Eight (II-1, II-2, II-4, III-1, III-2, III-8, III-13, and III-14) of them underwent surgery and one (III-12) has been scheduled for surgery. The mean age at surgery was 37 yr (range, 18 to 67). The youngest patient to present with MTC and lymph node metastases was

Table 1. Clinical characteristics of 9 gene carriers with medullary thyroid carcinoma

Case	Sex/age (yr)	Age at surgery (yr)	Preop. basal CT (pg/mL, <10 pg/mL)	Type of operation	Histology	Tumor	LN metastasis	TNM staging	Follow-up CT (pg/mL, <10 pg/mL)
II-1	F/72	48		TT with central and both lateral MRND	MTC	Rt 1.8 cm Lt 6.5 cm	Yes (14/38)	T3N1M0 Stage III	593.5
II-2	M/68	67	1,035.7	TT with central ND	MTC	Rt 0.3 cm Lt 2.5 cm	Yes (9/13)	T2N1M0 Stage III	1.3
II-4	M/59	42	1,000	TT with central ND	MTC	Rt 1.5 cm Lt 1 cm	No (0/6)	T2N0M0 Stage II	2.2
III-1	M/49	49	208.9	TT with central ND	MTC	Rt 0.3 cm Lt 1.2 cm	Yes (11/13)	T2N1M0 Stage III	
III-2	F/35	18		TT with central and left lateral MRND	MTC	Rt 3.5 cm Lt 0.6 cm	Yes (8/23)	T2N1M0 Stage III	
III-8	F/32	24	1,900	TT with central and left lateral MRND	MTC	Rt 0.8 cm Lt 3 cm	Yes (27/57)	T2N1M0 Stage III	133.7
III-12	F/28	Scheduled							
III-13	M/29	27	143.2	TT with central ND	MTC	Rt 1.0 cm Lt 0.5 cm	Yes (2/7)	T1N1M0 Stage III	0.2
III-14	M/27	19		TT with central and left lateral MRND	MTC	Lt 4.5 cm	Yes (13/38)	T3N1M0 Stage III	286.3

CT, calcitonin; M, male; F, female; TT, total thyroidectomy; MRND, modified radical neck dissection; ND, neck dissection; MTC, medullary thyroid cancer; Rt, right; Lt, left; LN, lymph node.

18-yr old. Seven patients had bilateral MTC. Seven patients with MTC had lymph node metastases including four lateral neck node metastases. Median follow-up period was 10 yr. There was no case with disease recurrence or death during follow-up. The brother of the index case (II-5) exhibited increased serum calcitonin in response to pentagastrin stimulation and underwent a total thyroidectomy at 45 yr of age; the histology examination showed normal thyroid tissue. He was confirmed not to have a RET mutation. Two family members positive for the RET mutation, a 37-yr-old man (III-3) and a 12-yr-old girl (IV-3), are asymptomatic and have no evidence of disease by thyroid ultrasound; they have refused further biochemical investigation.

DISCUSSION

We report a RET mutation in exon 10, codon 618 that causes substitution of a cysteine by a serine in the cysteine-rich domain of the RET receptor in a three-generation FMTC family composed of 30 members with 11 carriers. Approximately 85% of families with FMTC have an identifiable RET mutation (9). In general, FMTC patients have the same mutations as patients with MEN2A, but they are more evenly distributed among cysteines 618, 620, and 634 each accounting for 25% to 35% of the mutations. Five percent to 10% of FMTC patients have a mutation in an intercellular domain, that is, in codons 768, 804 or 891 (9, 12-15).

It has been established that specific RET mutations predict the phenotypic expression of the disease. It has been reported previously that mutations at codon 618 are associated with MEN2A, FMTC and Hirschsprung disease (HSCR1). Mutations in this codon are associated with low transforming activity of RET and may be associated with milder forms of the disease (16, 17). In the kindred that we have described here, all of the affected patients had MTC as the sole clinical finding and did not have any evidence of adrenal or parathyroid involvement on screening. The median age at surgery was 27 yr (range, 18 to 67). The median age at the time of the development of MTC, in this study in FMTC patients, was similar to the age reported for previous FMTC families. Among eight patients with MTC, seven had bilateral cancer and lymph node metastases including four lateral neck node metastases. The FMTC with cysteine RET mutations found in the Korean population is consistent with the clinical pattern reported worldwide; to date there have been no ethnic differences identified for FMTC.

Kouvaraki et al. (3) reported that RET mutations can be stratified into three groups (levels 1 to 3) based on the biological aggressiveness of MTC observed in patients with these mutations. Level II mutations are considered high risk, and correspond to MEN2A and some FMTC mutations, including codons 611, 618, 620, and 634. Carriers of high-risk RET mutations develop MTC starting from the age of one year (codon 634) and five years (codon 611, 618, and 620), lymph node metastasis from the first decade (codon 634) and the second decade (codon 611, 618, and 620), and distant metastases generally from the third decade (18). Current guide-

lines for the treatment of patients with level two, high-risk group mutations, recommend prophylactic total thyroidectomy before the age of five years. No agreement has been reached about the need for additional central lymph node dissection in patients with these mutations.

The 12-yr-old granddaughter (IV-3) in this family is being carefully monitored at the time of preparation of this report to determine the appropriate timing of thyroidectomy. Such surgery has not yet been performed because of the potential risks and complications associated with a thyroidectomy, the lack of radiological signs of development of MTC, and the possibility of slow growth of MTC associated with a codon 618 mutation. The other family members positive for the RET mutation, a 37-yr-old man (III-3), is asymptomatic and have no evidence of disease by thyroid ultrasound. We have recommended prophylactic thyroidectomy, however, to date they have refused the procedure.

In summary, we report here an extracellular mutation of the RET proto-oncogene that was identified in a large Korean family with FMTC. The mutation substituted a cysteine for a serine in codon 618 of exon 10 (C618S, TGC to AGC). Our results suggest that this genetic profile might be associated with usually aggressive clinical course with regional lymph node metastasis but late onset of MTC.

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