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. 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 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. 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

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 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. 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

© 2010 The Korean Academy of Medical Sciences.
This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

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 members of the family in Fig. 1. The 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 members of the family.
Familial Medullary Thyroid Cancer with Cys618Ser RET Germline Mutation

Familial Medullary Thyroid Cancer with Cys618Ser RET Germline Mutation

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

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
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-

| 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                  | 18                                | TT with central and both lateral MRND | MTC       | Rt 1.8 cm | Yes (14/38)  | T3N1M0      | 593.5                      |
| II-2 | M/68        | 67                  | 1,035.7                           | TT with central ND | MTC       | Rt 0.3 cm | Yes (9/13)   | T2N1M0      | 1.3                         |
| II-4 | M/59        | 42                  | 1,000                             | TT with central ND | MTC       | Rt 1.5 cm | No (0/6)     | T2N0M0      | 2.2                         |
| III-1| M/49        | 49                  | 208.9                             | TT with central ND | MTC       | Rt 0.3 cm | Yes (11/13)  | T2N1M0      | 133.7                      |
| III-2| F/35        | 18                  | 355.5                             | TT with central and left lateral MRND | MTC       | Rt 3.5 cm | Yes (8/23)   | T2N1M0      | 2.2                         |
| III-8| F/32        | 24                  | 1,900                             | TT with central and left lateral MRND | MTC       | Rt 0.8 cm | Yes (27/57)  | T2N1M0      | 133.7                      |
| III-12| F/28       | Scheduled            |                                   |                  | MTC       | Rt 1.0 cm | Yes (2/7)    | T1N1M0      | 0.2                         |
| III-13| M/29       | 27                  | 143.2                             | TT with central ND | MTC       | Lt 0.5 cm | T1N1M0      | Stage III  |               |
| III-14| M/27       | 19                  |                                   | TT with central and left lateral MRND | MTC       | Lt 4.5 cm | Yes (13/38)  | T3N1M0      | 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.
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 lack of radiological signs of development of MTC, and the risks and complications associated with a thyroidectomy, the surgery has not yet been performed because of the potential lack of radiological signs of development of MTC, and the risks and complications associated with a thyroidectomy, the surgery has not yet been performed because of the potential.

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.

REFERENCES

1. Alsanea O, Clark OH. Familial thyroid cancer. Curr Opin Oncol 2001; 13: 44-51.
2. Hemminki K, Dong C. Population-based study of familial medullary thyroid cancer. Fam Cancer 2001; 1: 45-9.
3. Kouvaraki MA, Shapiro SE, Perrier ND, Cote GJ, Gagel RF, Hoff AO, Sherman SI, Lee JE, Evans DB. RET proto-oncogene: a review and update of genotype-phenotype correlations in hereditary medullary thyroid cancer and associated endocrine tumors. Thyroid 2005; 15: 531-44.
4. Brandl ML, Gagel RF, Angeli A, Bilezikian JP, Beck-Peccoz P, Bordi C, Conte-Devolx B, Falchetti A, Gheri RG, Libroia A, Lips CJ, Lombardi G, Mannelli M, Pacini F, Ponder BA, Raue F, Skogseid B, Tamburrano G, Thakker RV, Thompson NW, Tomassetti P, Tonelli F, Wells SA Jr, Marx SJ. Guidelines for diagnosis and therapy of MEN type 1 and type 2. J Clin Endocrinol Metab 2001; 86: 5658-71.
5. Mulligan LM, Marsh DJ, Robinson BG, Schuffenecker I, Zedenius J, Lips CJ, Gagel RF, Takai SI, Noll WW, Fink M. Genotype-phenotype correlation in multiple endocrine neoplasia type 2: report of the International RET Mutation Consortium. J Intern Med 1995; 238: 343-6.
6. Fambon JR, Leight GS, Dilley WG, Baylin SB, Smallridge RC, Harrison TS, Wells SA Jr. Familial medullary thyroid carcinoma without associated endocrinopathies: a distinct clinical entity. Br J Surg 1986; 73: 278-81.
7. Eng C, Clayton D, Schuffenecker I, Lenoir G, Cote G, Gagel RF, van Amstel HK, Lips CJ, Nishisho I, Takai SI, Marsh DJ, Rosenberg BG, Frank-Raue K, Raue F, Xue F, Noll WW, Romci C, Pacini F, Fink M, Niederle B, Zedenius J, Nordenskjold M, Kommminoth P, Hendy GN, Mulligan LM. The relationship between specific RET proto-oncogene mutations and disease phenotype in multiple endocrine neoplasia type 2. International RET mutation consortium analysis. JAMA 1996; 276: 1575-9.
8. Ponder BA, Ponder MA, Coffey R, Pembrey ME, Gagel RF, Telenuis Berg M, Semple P, Easton DF. Risk estimation and screening in families with medullary thyroid carcinoma. Lancet 1988; 1: 397-401.
9. Mulligan LM, Eng C, Healey CS, Clayton D, Kwok JB, Gardner E, Ponder MA, Frilling A, Jackson CE, Lehnert H. Specific mutations of the RET proto-oncogene are related to disease phenotype in MEN 2A and FMTC. Nat Genet 1994; 6: 70-4.
10. Jimenez C, Dang GT, Schultz PN, El-Naggar A, Shapiro S, Barnes EA, Evans DB, Vassilopoulou-Sellin R, Gagel RF, Cote GJ, Hoff AO. A novel point mutation of the RET proto-oncogene involving the second intracellular tyrosine kinase domain in a family with medullary thyroid carcinoma. J Clin Endocrinol Metab 2004; 89: 3521-6.
11. Eng C, Mulligan LM, Smith DP, Healey CS, Frilling A, Raue F, Neumann HP, Ponder MA, Ponder BA. Low frequency of germline mutations in the RET proto-oncogene in patients with apparently sporadic medullary thyroid carcinoma. Clin Endocrinol (Oxf) 1995; 43: 123-7.
12. Bolino A, Schuffenecker I, Luo Y, Seri M, Silengo M, Tocco T, Chabrier G, Houdient C, Murat A, Schlumberger M. RET mutations in exons 13 and 14 of FMTC patients. Oncogene 1995; 10: 2415-9.
13. Berndt I, Reuter M, Saller B, Frank-Raue K, Groth P, Grussendorf M, Raue F, Ritter MM, Hoppner W. A new hot spot for mutations in the RET proto-oncogene causing familial medullary thyroid carcinoma and multiple endocrine neoplasia type 2A. J Clin Endocrinol Metab 1998; 83: 770-4.
14. Holstra RM, Fattoruso O, Quadro L, Wu Y, Libroia A, Verga U, Colantuoni V, Buys CH. A novel point mutation in the intracellular domain of the RET proto-oncogene in a family with medullary thyroid carcinoma. J Clin Endocrinol Metab 1997; 82: 4176-8.
15. Dang GT, Cote GJ, Schultz PN, Khorana S, Decker RA, Gagel RF. A codon 891 exon 15 RET proto-oncogene mutation in familial medullary thyroid carcinoma: a detection strategy. Mol Cell Probes 1999; 13: 77-9.
16. Takahashi M, Asai N, Iwashita T, Murakami H, Ito S. Molecular mechanisms of development of multiple endocrine neoplasia 2 by RET mutations. J Intern Med 1998; 243: 509-13.
17. Moers AM, Landsvater RM, Schaap C, Jansen-Schillhorn van Veen JM, de Valk IA, Blijham GH, Hoppener JW, Vroom TM, van Amsel HK, Lips CJ. Familial medullary thyroid carcinoma: not a distinct entity? Genotype-phenotype correlation in a large family. Am J Med 1996; 101: 635-41.
18. Machens A, Dallal H. Genotype-phenotype based surgical concept of hereditary medullary thyroid carcinoma. World J Surg 2007; 31: 957-68.