original article

RET codon 618 mutations in Saudi families with multiple endocrine neoplasia Type 2A and familial medullary thyroid carcinoma

Faiza Qari

From the Endocrine, King Abdulaziz University Hospital, Jeddah, Saudi Arabia

Correspondence: Dr. Faiza Qari · Endocrine, KAUH, PO Box 13042 Jeddah 21943, Saudi Arabia · T: 0505677905 F: 6408315 · faiza_qari@yahoo.com

Ann Saudi Med 2013; 33(2): 155-158

DOI: 10.5144/0256-4947.2013.155

BACKGROUND AND OBJECTIVES: Certain diseases such as multiple endocrine neoplasia (MEN) 2A, MEN 2B, familial and sporadic medullary thyroid carcinoma (MTC) and renal dysgenesis are related to abnormalities of the RET protein. Our aim was to evaluate the frequency of RET mutation in 10 Saudi families with MEN type 2A and familial MTC.

DESIGN AND SETTING: A cross-sectional prospective study of patients followed up at King Abdulaziz University Hospital and King Abdulaziz Medical City, Jeddah, between March 2001 and March 2011.

PATIENTS AND METHODS: Genomic DNA was isolated from peripheral blood leukocytes of all subjects by standard procedures. Exons 10, 11, 13, 14 and 16 of the RET proto-oncogene were analyzed by single-strand conformation polymorphism, direct DNA sequencing and/or restriction enzyme analysis.

RESULTS: We screened 79 subjects for the RET mutation. Of which 43 subjects had hereditary MTC were enrolled in this study. MEN type 2A was identified in 25 subjects; MTC was diagnosed in all 25 subjects (100%), pheochromocytoma in 13 subjects (52%) and hyperparathyroidism in 4 subjects (16%). The most frequent genotype in patients with MEN 2A syndrome was a codon 618 mutation (46.6%), followed by a codon 634 mutation (44.2%). Among the 5 families with MEN 2A, 3 had a mutation at codon 634, whereas 2 had a mutation at codon 618.

CONCLUSION: The most frequent RET proto-oncogene mutation in our series was in codon 618 (exon 10).

Medullary thyroid carcinoma (MTC) originates from para-follicular (C) cells or calcitonin-producing C cells, and it represents 5–10% of well-differentiated thyroid tumours, 0.4–1.4% of all thyroid nodules, and less than 1% of thyroid nodules determined by autopsy.[1] In patients with familial medullary thyroid carcinoma (FMTC), only the thyroid gland is affected, whereas in patients with multiple endocrine neoplasia (MEN) 2A, MTC, pheochromocytoma and/or primary hyperparathyroidism may occur.[2] In contrast, when patients with MEN 2B have MTC, they tend to develop pheochromocytoma, ganglioneuromas of the digestive tract, mucosal neuromas and/or skeletal abnormalities.[3] MTC occurs either sporadically or is inherited as part of the MEN type 2 syndromes (MEN 2A, MEN 2B and FMTC).[4]

Approximately 40% to 70% of sporadic cases and up to 95% of inherited cases harbour RET proto-oncogene mutations.[5]

The RET proto-oncogene was identified during analysis of DNA polymorphisms in chromosome 10 in large MEN 2 genealogies in 1986.[6] The RET protein, a product of the RET proto-oncogene, is a receptor-type tyrosine kinase of neural crest origin that is expressed in the endocrine tissue. RET abnormalities are related to the pathogenesis of several diseases such as MEN 2A, MEN 2B, familial and sporadic MTC, congenital aganglionic megacolon, papillary thyroid cancer and renal dysgenesis. RET is the only gene known to be associated with MEN 2, and hereditary mutations of this proto-oncogene have been extensively studied.[7] The management of MEN 2 by using DNA sequencing data represents an excellent example of how molecular DNA diagnosis may improve clinical management.[8] In the present study, we evaluated

the frequency of RET mutation in 10 Saudi families with MEN 2A and FMTC.

PATIENTS AND METHODS

This was a prospective cross-sectional study that aimed to evaluate the frequency of the RET mutation genotype in subjects with MEN 2A or FMTC who were followed up for thyroid cancer at King Abdulaziz University Hospital and King Abdulaziz Medical City, Jeddah, between March 2001 and March 2011. Written informed consent was obtained from all the participants prior to their inclusion in the study. Ethical approval for the study was granted by the Biomedical Ethics Research Committee of King Abdulaziz University.

Ten unrelated Saudis with germline mutation of the RET proto-oncogene or MTC diagnosed by immunohistochemistry were included in the study. Pheochromocytoma and hyperparathyroidism were detected by extensive testing of all participants and their relatives who were at risk of developing FMTC. The medical history of all participants was reviewed, and physical examination and biochemical measurements of fasting serum calcium, basal plasma calcitonin levels, plasma parathyroid hormone (1–84), 24-h urinary excretion of catecholamines and metabolites, and DNA analysis were performed. Genomic DNA was isolated from peripheral blood leukocytes by using established standard techniques. Exons 10, 11, 13, 14, 15 and 16 of the RET proto-oncogene were analyzed by the established single-strand conformation polymorphism technique, direct DNA sequencing and restriction enzyme analysis. A mutation was identified in all kindred patients with documented germline transmission of MTC.

RET genetic analysis

The RET gene exons 10, 11, 13, 14, 15 and 16 were analyzed in all subjects by polymerase chain reaction (PCR) and DNA sequencing following the salting-out method,[9] which is an established technique used at the King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia. In this technique, 100 ng of DNA is amplified in a final volume of 25 µL containing 1 µmol/L of each oligonucleotide primer, 10 mmol/L Tris-HCl (pH 8.3), 2.5 mmol/L $MgCl^2$, and 1 U Taq polymerase (Roche Molecular Systems, Inc., Branchburg, NJ). The PCR was started with an initial denaturation step at 95°C for 1 min, followed by 35 cycles of 1 min each at 65°C, 72°C and 95°C, and completed for another 5 min at 72°C. The amplified DNA was analyzed on a 2% agarose gel and purified with the Qiagen Quickspin kit. The presence of mutation was confirmed by direct sequencing of the purified PCR product by using the signal method in an automated sequencer according to the manufacturer's instructions (dRhodamine Terminator Cycle Sequencing Ready Reaction Kit, Applied Biosystems Inc., Foster City, CA, USA).

Classification of MEN 2 as suggested by the international consensus on MEN 1

This classification is based on the clinical pictures of affected patients with MEN 2.[10]

Phenotypic classification of MEN 2

Multiple endocrine neoplasia 2 A (1): Families with medullary thyroid carcinoma, pheochromocytoma and hyperparathyroidism phenotypes

Multiple endocrine neoplasia 2A (2): Families with medullary thyroid carcinoma and pheochromocytoma phenotypes in at least one at-risk relative.

Multiple endocrine neoplasia 2A (3): Families with medullary thyroid carcinoma and hyperparathyroidism phenotypes in at least one at-risk relative.

Multiple endocrine neoplasia 2B: Families with medullary thyroid carcinoma, associated or not with pheochromocytoma and muscular/skeletal abnormalities and mucosal neuromas.

Familial medullary thyroid carcinoma: Families with only medullary thyroid carcinoma phenotype in at least 4 at-risk relatives.

Others: Families with less than 4 relatives with medullary thyroid carcinoma, pheochromocytoma and hyperparathyroidism phenotypes. Partially documented families are included in this group.

The Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

RESULTS

A total of 79 subjects from 10 unrelated Saudi families were screened for mutations of the RET proto-oncogene; these study subjects included 34 men and 45 women, (male: female ratio=0.7:1). Forty-three subjects with hereditary MTC who were positive for the RET proto-oncogene mutation were enrolled in the study.

Of the study subjects, 30 patients were aged 12–65 years at the time when they underwent total thyroidectomy; 10 patients had undergone surgery for pheochromocytoma, whereas 3 had a history of surgery for hyperparathyroidism. Furthermore, 16 of the 43 patients with hereditary MTC did not have clinical evidence of disease on molecular screening but were considered to

be at-risk because of an affected relative.

The diagnosis of MTC, pheochromocytoma and parathyroid hyperplasia was confirmed by postoperative histopathology. MEN and FMTC were identified in 5 families each. Amongst the 25 patients identified with MEN 2A, MTC was diagnosed in all 25 patients (100%); pheochromocytoma, in 13 patients (52%); and hyperparathyroidism, in 4 patients (16%). The onset of MTC in patients with MEN 2A was earlier than in cases with pheochromocytoma and hyperparathyroidism.

The most frequent genotype of MEN 2A syndrome was located in codon 618 mutation (46.6%), followed by a codon 634 mutation (44.2%) (**Figure 1**). The screening of exons 10, 11, 13, 14 and 16 was negative for RET mutations in one family. Amongst the 5 families with MEN 2A, 2 had mutations at codon 618 in exon 10, whereas 3 had mutations at codon 634 in exon 11. Two families amongst the 4 families with FMTC had mutations at codon 618 in exon 10; the mutation was located in codon 634 (exon 11) in one subject and in codon 611 (exon 10) in another subject (**Table 1**).

DISCUSSION

Mutations that cause activation of RET have been well characterized, and several groups have studied the disease phenotype–genotype relationships. The frequency of specific RET mutations in MEN 2A phenotypes varies between countries,[11,12] suggesting that the occurrence of these mutations may be influenced by genetic factors. Recently, the results of a study carried out by the International RET Mutation Consortium showed that 86% of MEN 2A families had a mutation at codon 634; the resulting amino acid change was most frequently C634R (in 52% of the subjects), followed by C634Y (in 26% of the subjects). On the other hand, in families with FMTC, the most frequent mutation was at codon 634, giving rise solely to the C634Y amino acid change.[13]

In our series, the most frequent genotype was the MEN 2A syndrome with codon 618 mutation in 46.6% of the subjects. This finding is contrary to those of the International RET mutation Consortium analysis, which showed that the mutation at codon 634 was found in 86% of all subjects with MEN 2A.[14] In the current study, the family with FMTC presented the C634Y mutation, the most prevalent codon 634-specific mutation associated with this phenotype in the RET Consortium.

Families with hereditary MTC exhibited a highly

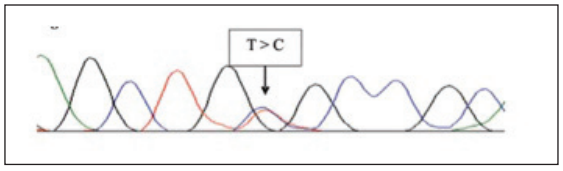

Figure 1. T-to-C substitution (TGC-CGC) in codon 634 (exon 11) in the RET proto-oncogene, leading to a Cys634Arg mutation.

Table 1. Summary of Patients with MEN Type 2A and familial medullary thyroid carcinoma.

Family members	Sex Male: female	Pathology	Exon	Mutation	Number of positive/total number of members	Total number of members screened
I	8:3	MEN 2A	10	618	6:11	11
II	2:4	MEN 2A	10	C618	4:6	6
III	3 :5	FMTC	10	C618s	7:8	8
IV	5 :7	MEN 2A	11	C634y	7:12	12
V	5:3	MEN 2A	11	634	5:8	8
VI	2:8	FMTC	11	634	4:10	10
VII	1:3	FMTC	10	618	3:4	4
VIII	2:4	MTC	Negative	Negative	0:6	4
IX	2:4	FMTC	10	C611	4:6	6
X	4:6	MEN 2A	11	634	3:7	10
	Total Number 34:45					**Total Number 43:79**

Abbreviations: MEN, multiple endocrine neoplasia; MTC, medullary thyroid carcinoma; FMTC, familial medullary thyroid carcinoma

variable disease presentation and a more aggressive disease, as demonstrated by more frequent distant metastases at diagnosis. They did not, however, develop metastasis at an earlier age.[15] In contrast, the thyroid carcinoma genotype appears to have a benign indolent behaviour, with low potential to spread in some individuals.[16,17] On the basis of these observations, we suggest that there is no need to speed up prophylactic thyroidectomy in subjects with a heterozygous mutation in codon 618.[18,19]

Our study had a few limitations in that our sample size was small. It is possible that there was interference of other hereditary molecular genotypes when unit genotype was used in the analysis of individuals who belonged to the same kindred. However, the high frequency of mutations in codon 618 in our study subjects may reflect the different hereditary factors in our study population compared with others. Detailed information of the RET mutation and its clinical implications remain to be elucidated. Further large multicenter studies should be conducted in Saudi patients in order to completely understand the disease mechanisms and to clarify the process leading to development of the RET codon 618 mutant genotype in Saudi families with hereditary MEN 2A and FMTC.

In conclusion, we reported the frequency of RET proto-oncogene mutations in a sample of 10 unrelated Saudi families with hereditary MTC and MEN 2A. The most frequent RET proto-oncogene mutation was found to be in codon 618 (exon 10) in our series.

REFERENCES

1. Pinchera A, Elisei R. Medullary thyroid cancer: diagnosis and management. In: Mazzaferri EL, Ujjal K, Mallick UK, Kendall-Taylor P, editors. Practical management of thyroid cancer. London: Springer-Verlag; 2006. p. 255–79.

2. Gimm O. Multiple Endocrine Neoplasia Type 2: Clinical Aspects. In: Dahia PLM, Eng C. Genetic Disorders of Endocrine Neoplasia. 1st ed. Philadelphia: Front Horm Res Basel Karger; 2001. p. 103-30.

3. Hoff AO, Cote GJ, Gagel RF. Multiple endocrine neoplasias. Annu Rev Physiol. 2000;62:377-411.

4. Machens A, Gimm O, Hinze R, Hoppner W, Boehm BO, Dralle H. Genotype-phenotype correlations in hereditary medullary thyroid carcinoma:oncological features and biochemical properties. J Clin Endocrinol Metab. 2001;86(3):1104–9.

5. Hansford JR, Mulligan LM. Multiple endocrine neoplasia type 2 and RET: from neoplasia to neurogenesis. J Med Genet. 2000;37(11):817-27.

6. Gagel RF. RET proto-oncogene mutations and endocrine neoplasia – A story interwined with neuronal crest differentiation. Endocrinology. 1996;137:1509-11.

7. Toledo SP, dos Santos MA, Toledo Rde A, Lourenço DM Jr. Impact of RET proto-oncogene analysis on the clinical management of multiple endocrine neoplasia type 2. Clinics (Sao Paulo). 2006 Feb;61(1):59-70.

8. Eng C. RET proto-oncogene in the development of human cancer. J Clin Oncol. 1999;17(1): 380–93.

9. Marx SJ. Molecular genetics of multiple endocrine neoplasia types 1 and 2. Nat Rev Cancer. 2005;5(5)1:367-75.

10. Brandi ML, Gagel RF, Angeli A, Bilezikian JP, Beck-Peccoz P, Bordi C, et al. Guidelines for diagnosis and therapy of MEN type 1 and type 2. J Clin Endocrinol Metab. 2001;86(12):5658-71.

11. Erdogan MF, Gürsoy A, Ozgen G, Cakir M, Bayram F, Ersoy R, et al. Ret proto-oncogene mutations in apparently sporadic Turkish medullary thyroid carcinoma patients: Turkmen study. J Endocrinol Invest. 2005;28(9):806-9.

12. Chung YJ, Kim HH, Kim HJ, Min YK, Lee MS, Lee MK, et al. RET proto-oncogene mutations are restricted to codon 634 and 618 in Korean families with multiple endocrine neoplasia 2A. Thyroid. 2004;14(10):813-8.

13. Feldman GL, Edmonds MW, Ainsworth PJ, Schuffenecker I, Lenoir GM, Saxe AW, et al. Variable expressivity of familial medullary thyroid carcinoma (FMTC) due to a RET V804M (GTG ---->ATG) mutation. Surgery. 2000;128(1):93–8.

14. Puñales MK, Graf H, Gross JL, Maia AL. RET codon 634 mutations in multiple endocrine neoplasia type 2: variable clinical features and clinical outcome. J Clin Endocrinol Metab. 2003;88(6):2644-9.

15. Szinnai G, Meier C, Komminoth P, Zumsteg UW. Review of multiple endocrine neoplasia type 2A in children: therapeutic results of early thyroidectomy and prognostic value of codon analysis. Pediatrics. 2003;111(2):132-9.

16. Lombardo F, Baudin E, Chiefari E, Arturi F, Bardet S, Caillou B, et al. Familial medullary thyroid carcinoma: clinical variability and low aggressiveness associated with RET mutation at codon 804. J Clin Endocrinol Metab. 2002;87(4):1674-80.

17. Skinner MA, Moley JA, Dilley WG, Owzar K, Debenedetti MK, Wells SA Jr. Prophylactic thyroidectomy in multiple endocrine neoplasia type 2A. N Engl J Med. 2005;353:1105-13.

18. Sanso GE, Domene HM, Garcia R, Pusiol E, de M, Roque M, et al. Very early detection of RET proto-oncogene mutation is crucial for preventive thyroidectomy in multiple endocrine neoplasia type 2 children: presence of C-cell malignant disease in asymptomatic carriers. Cancer. 2002;94(2):323-30.

19. Hansen HS, Torring H, Godballe C, Jäger AC, Nielsen FC. Is thyroidectomy necessary in RET mutations carriers of the familial medullary thyroid carcinoma syndrome? Cancer 2000;89(4):863–7.