A *RET* C634R Mutation in a Thai Female with Multiple Endocrine Neoplasia Type 2A

SARAT SUNTHORNYOTHIN, MD*,
THIVARATANA SINTHUWIWAT, BSc**,
VORASUK SHOTELERSUK, MD**

Abstract

Multiple endocrine neoplasia type 2A (MEN 2A) is an autosomal dominant disorder characterized by medullary thyroid carcinoma, pheochromocytoma and primary hyperparathyroidism. The first tumor is usually a medullary thyroid carcinoma. MEN 2A is caused by mutations in the *RET* proto-oncogene. The detection of mutations in the gene has important diagnostic and therapeutic impacts. Genetic testing of at-risk family members allows one to identify individuals carrying the mutant alleles with very high specificity and sensitivity. Subsequently, total thyroidectomy, recommended at 5 years of age, can be performed in a prophylactic attempt.

The authors performed a molecular analysis to identify a mutation in a Thai woman with MEN 2A. She was found to be heterozygous for 1900T>C (C634R). The patient had two daughters who were not found to carry the mutation.

The newly available genetic test for patients with MEN 2A in Thailand makes possible accurate DNA-based diagnosis of their at-risk family members before development of the disease, which has important therapeutic impacts for them.

Key word : Multiple Endocrine Neoplasia, RET, Mutation Analysis

SUNTHORNYOTHIN S,
SINTHUWIWAT T, SHOTELERSUK V
J Med Assoc Thai 2003; 86 (Suppl 2): S472-S476

* Department of Internal Medicine,
** Department of Pediatrics, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand.

Multiple endocrine neoplasia (MEN) is a hereditary cancer syndrome characterized by the occurrence of tumors involving two or more endocrine glands within a single patient. Two major forms of MEN are recognized and referred to as type 1 (MEN1) and type 2 (MEN2A and MEN2B)[1]. MEN 2A is an autosomal dominant disorder characterized by medullary thyroid carcinoma, pheochromocytoma and primary hyperparathyroidism (OMIM no. 171400). Patients have been reported worldwide including Asia [2-4] and Thailand[5,6]. Germ-line mutations of the *RET* proto-oncogene have been identified as the underlying cause of the disorder[7]. The gene was mapped to chromosome 10q11.2, has 21 exons, and encodes a receptor tyrosine kinase that is expressed in derivatives of neural crest cells. A molecular diagnosis of patients with MEN 2A makes DNA testing of at -risk family members available. It, unlike biochemical tests, permits the unambiguous identification of MEN 2A gene carriers[8]. The identification of a mutation has important implications for clinical management, including lifesaving prophylactic treatment. The authors performed a molecular genetic test to identify a mutation in *RET* in a Thai woman with MEN 2A. This is the first published genetic analysis of MEN 2A in Thailand.

MATERIAL AND METHOD

Case report

A 42-year-old Thai woman was referred to the King Chulalongkorn Memorial Hospital for management of congestive heart failure, uncontrolled hypertension, severe hyperglycemia and bilateral adrenal masses. Details of the patient was previously published[5]. In summary, she was found to have bilateral pheochromocytoma, (Fig. 3) primary hyperparathyroidism and medullary thyroid carcinoma. She underwent bilateral adrenalectomy and subsequently, total thyroidectomy and parathyroidectomy. Her 24-hour urinary metanephrines post-operatively returned to normal range. Her blood pressure and glucose level have been under control with minimal medications. She has two daughters, aged 13 and 6 years.

Mutation analysis

After informed consent was obtained, DNA was extracted from the patient and her two daugthers by a standard method. *RET* exon 11 was polymerase chain reaction (PCR) amplified using 4 µl of gDNA, 1XPCR buffer (Promega, Wisconsin, USA), 1.5 mM $MgCl_2$, 200 µM dNTPs, 0.25 µM of each primer, and 0.4 U Taq DNA polymerase in a total volume of 20 µl. The primer sequences were 5'- GCCATGAGGC AGAGCATA-3' (RET11F) and 5' TGGGGAGGCCA GGGGATCTT-3' (RET11R), yielding a 384-bp product. An initial denaturation step of 94°C for 5 min was followed by 40 PCR cycles, each with a denaturation step of 94°C for 45 s, an annealing of 60°C for 45 s, and an extension of 72°C for 45 s. Ampiification cycles were followed by an elongation step of 72°C for 10 min.

PCR products were cloned using pGEM®-T Easy Vector System I (Promega, Wisconsin, USA), according to the manufacturer's recommendations. The PCR products and two plasmid inserts were then sent for sequencing at the National Science and Technology Development Agency, Bangkok, Thailand.

The mutation was confirmed by cleavage of the PCR product with *Hha* I restriction endonuclease (New England BioLabs, Beverly, MA, USA). Twelve µl of PCR product was incubated with the enzyme for 16 h at 37°C.

RESULTS

A heterozygous T>C transition at nucleotide 1900 was identified in *RET* exon 11 of the patient from direct sequencing of the PCR product. One of the

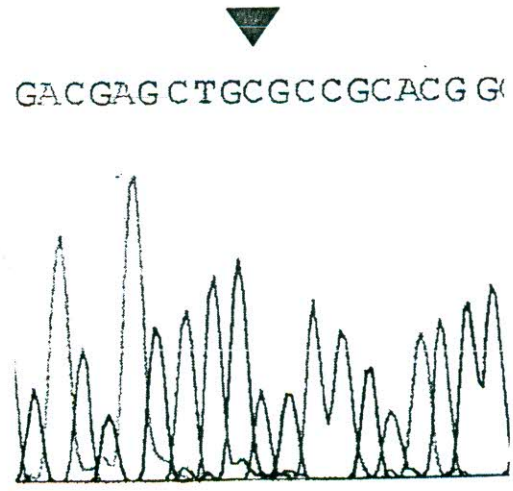

Fig. 1. The sense sequence electropherograms of the plasmid insert of the proband. The arrow head indicates the substitution of a C for the normal T.

Fig. 2. **Restriction enzyme detection of the C634R mutation in MEN 2A. Lanes 1 and 11 represents a marker (M). Lane 2 serves as a negative control (-ve). Lanes 3 and 4 were of a normal control (C); lanes 5 and 6 the proband's elder daughter (D1); lanes 7 and 8 the proband; lanes 9 and 10 the proband's younger daughter (D2). Lanes 3, 5, 7, and 9 were PCR products without adding restriction enzymes (N) and only the undigested 384 bp bands were presented. Lanes 4, 6, 8, and 10 were PCR products mixed with restriction endonuclease enzyme *Hha* I (E). The new smaller band in lane 8 demonstrates that the proband is heterozygous for the 1900T>C mutation.**

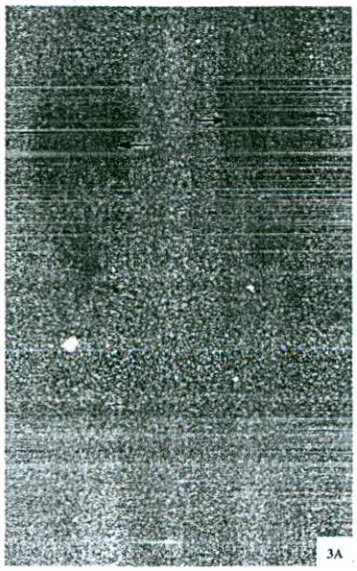

Fig. 3A. **^{131}I MIBG shows abnormal accumulation of tracer at both adrenal glands, more on the right side (thin arrows).**

Fig. 3B. **MRI demonstrates well-marginated mixed solid and partly cystic bilateral adrenal masses measuring 10 x 5 x 5.5 cm and 6 x 4 x 3 cm right and left adrenal respectively (thick arrows).**

clones showed the mutated sequence (Fig. 1), while the other was normal (data not shown). Restriction enzyme digestion of the patient and her two daughters revealed the pattern of mutation only in the proband (Fig. 2). The mutation is expected to result in subsequent substitution of an arginine for the normal cysteine at codon 634 (C634R).

DISCUSSION

The presented patient had medullary thyroid carcinoma, pheochromocytoma and primary hyperparathyroidism, which are typical manifestations of MEN 2A. She was previously described but not molecularly characterized[5]. No mutation analysis was performed either in the other reported Thai patient

with MEN 2A[6].

MEN 2A is transmitted in an autosomal dominant manner with virtually 100 per cent penetrance [9]. The first tumor is usually medullary thyroid carcinoma, which may occur very early in life[10]. Its only potentially curative treatment is surgical removal of all thyroid tissue, a goal not commonly achieved in patients with clinically manifest carcinoma. The detection of mutations in the *RET* gene has important diagnostic and therapeutic impacts. Genetic testing of family members at risk allows one to identify individuals carrying mutant alleles with very high specificity and sensitivity[11]. Subsequently, total thyroidectomy can be performed in a prophylactic attempt. Currently, the procedure is recommended at 5 years of age[12,13]. Individuals who carry the mutation are also subjected to clinical and laboratory surveillance for pheochromocytoma and primary hyperparathyroidism.

The authors developed a DNA test to identify a mutation in a Thai woman with MEN 2A. She was found to be heterozygous for 1900T>C (C634R). The C634R mutation is one of the most frequent changes found in patients with MEN 2A[14]. It is a gain-of function mutation[15]. In addition, it is associated with the earliest development of the thyroid carcinoma compared to mutations at codons 618, 620, and 804[16]. The presented patient has two daughters. If either of them carried the mutation, total thyroidectomy would have been recommended. Fortunately, only normal sequence was found after their blood samples were tested for the mutation.

In summary, the authors developed a genetic test for patients with MEN 2A in Thailand, making accurate DNA-based diagnosis of their at-risk family members possible before development of the disease. Identification of individuals carrying mutant alleles has an important therapeutic impact.

ACKNOWLEDGEMENT

The authors wish to thank Dr. Pimolrat Techopitayakul for taking care of the family. This work was supported by the Thailand Research Fund; Contract grant number: RSA/05/2544, and The Development Grants for New Faculty/Researchers, Chulalongkorn University.

(Received for publication on April 6, 2003)

REFERENCES

1. Brandi ML, Gagel RF, Angeli A, et al. Guidelines for diagnosis and therapy of MEN type 1 and type 2. J Clin Endocrinol Metab 2001; 86: 5658-71.
2. Iihara M, Yamashita T, Okamoto T, et al. A nationwide clinical survey of patients with multiple endocrine neoplasia type 2 and familial medullary thyroid carcinoma in Japan. Jpn J Clin Oncol 1997; 27: 128-34.
3. Lee MS, Hwang DY, Kim YH, et al. Mutations of ret proto-oncogene in 3 Korean families with MEN 2A: Clinical use of new restriction sites for genetic diagnosis. Endocr J 1998; 45: 555-61.
4. Zirie M, Mohammed I, El-Emadi M, Haider A. Multiple endocrine neoplasia type iia: Report of a family with a study of three generations in qatar. Endocr Pract 2001; 7: 19-27.
5. Niruthisard S, Chatrkaw P, Laornual S, Sunthornyothin S, Prasertsri S. Anesthesia for one-stage bilateral pheochromocytoma resection in a patient with MEN type IIa: Attenuation of hypertensive crisis by magnesium sulfate. J Med Assoc Thai 2002; 85: 125-30.
6. Seeherunvong T, Churesigaew S, Hemsrichart V. Multiple endocrine neoplasia type IIa: A case report. J Med Assoc Thai 1998; 81: 789-93.
7. Mulligan LM, Kwok JB, Healey CS, et al. Germline mutations of the RET proto-oncogene in multiple endocrine neoplasia type 2A. Nature 1993; 363: 458-60.
8. Lips CJ, Landsvater RM, Hoppener JW, et al. Clinical screening as compared with DNA analysis in families with multiple endocrine neoplasia type 2A. N Engl J Med 1994; 331: 828-35.
9. Ponder BA, Ponder MA, Coffey R, et al. Risk estimation and screening in families of patients with medullary thyroid carcinoma. Lancet 1988; 1: 397-401.
10. Nguyen L, Niccoli-Sire P, Caron P, et al. Pheochromocytoma in multiple endocrine neoplasia type 2: A prospective study. Eur J Endocrinol 2001; 144: 37-44.
11. Marsh DJ, Mulligan LM, Eng C. RET proto-oncogene mutations in multiple endocrine neoplasia

type 2 and medullary thyroid carcinoma. Horm Res 1997; 47: 168-78.
12. Skinner MA, DeBenedetti MK, Moley JF, Norton JA, Wells SA Jr. Medullary thyroid carcinoma in children with multiple endocrine neoplasia types 2A and 2B. J Pediatr Surg 1996; 31: 177-81; discussion 181-2.
13. Gill JR, Reyes-Mugica M, Iyengar S, Kidd KK, Touloukian RJ, Smith C, Keller MS, Genel M. Early presentation of metastatic medullary carcinoma in multiple endocrine neoplasia, type IIA: implications for therapy. J Pediatr 1996;129:459-64.
14. Eng C, Clayton D, Schuffenecker I, et al. 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.
15. Santoro M, Melillo RM, Carlomagno F, Fusco A, Vecchio G. Molecular mechanisms of RET activation in human cancer. Ann N Y Acad Sci 2002; 963: 116-21.
16. 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: E132-9.

การกลายพันธุ์ชนิด C634R ในจีนส์ RET ในหญิงไทยที่เป็น Multiple endocrine neoplasia type 2A

สารัช สุนทรโยธิน, พบ,*
*ทิวารัตน์ สินธุวิวัฒน์, วทบ**, วรศักดิ์ โชติเลอศักดิ์, พบ***

Multiple endocrine neoplasia type 2A (MEN 2A) เป็นโรคที่ถ่ายทอดแบบจีนส์เด่นบนออโตโซม มีลักษณะสำคัญคือ medullary thyroid carcinoma, pheochromocytoma และ primary hyperparathyroidism โดยมะเร็งชนิดที่เกิดขึ้นก่อน มักเป็น medullary thyroid carcinoma โรคนี้เกิดจากการกลายพันธุ์ในจีนส์ RET การตรวจการกลายพันธุ์มีประโยชน์ทั้งในด้านการวินิจฉัยและการรักษา วิธีดังกล่าวสามารถใช้วินิจฉัยผู้ที่มีการกลายพันธุ์ได้อย่างแม่นยำ ซึ่งผู้ที่พบว่ามีการกลายพันธุ์ควรได้รับคำแนะนำให้ตัดต่อมไทรอยด์ออกทั้งหมดก่อนอายุ 5 ปี เพื่อป้องกันการเกิด medullary thyroid carcinoma

คณะผู้วิจัยได้พัฒนาวิธีการวิเคราะห์หาการกลายพันธุ์ในผู้ป่วยหญิงไทยรายหนึ่งที่เป็น MEN 2A และพบว่าผู้ป่วยมีการกลายพันธุ์ชนิด heterozygous 1900T>C (C634R) โดยบุตรสาวทั้งสองของผู้ป่วยไม่มีการกลายพันธุ์ดังกล่าว

การพัฒนาการตรวจหาการกลายพันธุ์ในจีนส์ RET สำหรับผู้ป่วย MEN 2A ในประเทศไทย ทำให้สามารถใช้วิธีการดังกล่าวมาวินิจฉัยสมาชิกในครอบครัวของผู้ป่วยได้อย่างแม่นยำ ซึ่งจะมีผลในด้านการป้องกันและรักษาต่อไป

คำสำคัญ : มัลติเปิ้ล เอนโดครีน นีโอเพลเซีย, อาร์อีที, การตรวจหาการกลายพันธุ์

สารัช สุนทรโยธิน, ทิวารัตน์ สินธุวิวัฒน์, วรศักดิ์ โชติเลอศักดิ์
จดหมายเหตุทางแพทย์ ฯ 2546; 86 (ฉบับพิเศษ 2): S472–S476

* ภาควิชาอายุรศาสตร์,
** ภาควิชากุมารเวชศาสตร์, คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย, กรุงเทพ ฯ 10330