CASE REPORTS

GENETIC TESTING IDENTIFIES THE POTENTIAL RISK OF MULTIPLE ENDOCRINE NEOPLASIA IN A VIETNAMESE FAMILY

Nguyen Hai Ha 1,2,*, Nguyen Thi Thanh Hoa 1, Pham Thi Dung 3, Nguyen Huy Binh 4, Nguyen Dang Ton 1,2

1Institute of Genome Research, Vietnam Academy of Science and Technology, Hanoi, Vietnam
2Graduate University of Science and Technology, Vietnam Academy of Science and Technology, Hanoi, Vietnam
3E Hospital, Hanoi, Vietnam
4Hanoi Medical University, Vietnam

*To whom correspondence should be addressed. E-mail: nguyenhaiha@igr.ac.vn

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SUMMARY

Multiple endocrine neoplasia type 2A (MEN2A) is a rare syndrome characterized by the presence of medullary thyroid carcinoma, pheochromocytoma, hyperparathyroidism and sometimes cutaneous lichen amyloidosis. This syndrome is caused by a germline activation mutation in the rearranged during transfection (RET) proto-oncogene transmitted by an autosomal dominant inheritance. In this study, we reported a rare case of a 44-year-old man from Vietnam with medullary thyroid carcinoma and pheochromocytoma as the symptom of MEN2A. Genetic testing indicated a nucleotide substitution located in exon 11 of the RET proto-oncogene (c.1900T>C, p.C634R), which was reported as a known pathogenic mutation of MEN2A. Further genetic tests on the other family members found the same mutation in his daughter (currently 14 years-old) and his son (currently 8 years-old). Although these 2 children do not yet have any manifestations of MEN2A, this data emphasizes their high risks of this disease. Therefore, this case draws attention to the importance of genetic counselling in C634R carriers, as well as rigorous follow-up appointments to reduce incidence and mortality since the mutation is classified as a high-risk group within the medullary thyroid carcinoma guidelines.

Keywords: Multiple Endocrine Neoplasia 2A, RET Proto-oncogene, p.C634R mutation

INTRODUCTION

Multiple Endocrine Neoplasia (MEN) type 2 syndromes are rare endocrinopathies characterized by the combined occurrence of endocrine tumors and abnormalities in non-endocrine tissues. They have been described in almost all ethnic groups throughout the world. Based on an analysis of SEER data, MEN2A is by far the common case, with an incidence of 1 patient in 2 million (Wells et al., 2015), and about 95% of the patients with MEN2 are classified as type 2A (Wells et al., 2013). Nearly all patients with MEN2A have either C-cell hyperplasia (CCH) or medullary thyroid cancer (MTC), around 50% have pheochromocytoma (Pheo), and 20-30% have hyperparathyroidism (HPT). MEN2 syndromes have autosomal dominant transmission patterns, which means children of affected individuals have a 50% chance of inheriting the genetic abnormality. The penetrance is virtually 100% by biochemical screening. However, only 60-70% develop clinically apparent syndromes (Horiuchi et al.,...
About 25% of patients with MTC have one of these familial syndromes. Patients who are diagnosed with MTC and have a negative family history need to be screened for MEN2, and a percentage of 3% to 7% was found to have a hereditary syndrome (Wells et al., 2013).

MEN2 is caused by germline gain-of-function mutations of the RET proto-oncogene that are located at the centromeric region of chromosome 10 (10q11-2). The RET proto-oncogene gene is a transmembrane tyrosine kinase with a long extracellular domain, a single transmembrane region, and two cytoplasmic tyrosine kinase domains. The oncogene contains 21 exons spanning more than 60 kb of genomic DNA. All classic MEN2A families studied thus far possessed germline mutations of the RET proto-oncogene, but a few families with MEN2B or FMTC did not have such mutations. These investigations showed that these mutations resulted in the activation of estrogen-responsive elements and increased RET expression through a novel mechanism (Hughes et al., 2000). Mutations that affect the cysteine-rich extracellular domain encoded in RET exons 10–15 have been found to associate with MEN2A, two mutations in RET exons 15 and 16 associated with MEN2B, and mutations at certain codons in various exons of RET (5, 8, 11, 12, 13, 14, 15, 16) associate with familial medullary thyroid carcinoma (FMTC) (Kouvaraki et al., 2005). However, the C634R mutation in exon 11 of the RET is a rare mutation and has been found to mainly associate with MEN2A (Hyde et al., 2017).

In this study, we report an exceptional case of MEN2A in a 44-year-old man diagnosed with medullary thyroid carcinoma and pheochromocytoma. This patient is found to possess germline C634R (c.1900T > C) RET mutation.

CASE DESCRIPTION

Proband (I:1) is a 44-year-old male who was admitted to Hanoi Medical University Hospital in August 2018 for the elucidation of the cause of dizziness and palpitation, which had been occurring for about half a year. Biochemical examinations including quantitation of total protein, albumin, total cholesterol, and triglyceride, as well as immunological testing of free thyroxine, thyroid stimulating hormone, C-peptide, cortisol, and adrenocorticotropic hormone were normal. However, catecholamine, serum calcitonin level in his plasma and 24-h urine were elevated, suggesting an abnormality in the thyroid. A computed tomography (CT) scan revealed bilateral adrenal and thyroid masses. The thyroid scintigraphy imaging provided localization of abnormal radiopharmaceutical accumulations.

Selective screening for the RET proto-oncogene at exons 5, 8, 10, 11, and 13-16 was performed using genomic DNA extracted from the peripheral blood leukocytes of the patient. A heterozygous, pathogenic germline mutation c.1900T>C was detected at exon 11 of the RET gene (Gene ID: 5979; OMIM 164761) by direct sequencing using an ABI 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). This transversion substituted arginine with cysteine at codon 634 (p.Cys634Arg) (Figure 1A). It further confirmed that the index patient I:1 had TC with the subtype MEN2A, and treatment via both adrenalectomy and thyroidectomy was necessary.

Since I:1 harbors the C634R germline mutation, all his family members were asked for RET mutation screening, regardless of clinical symptoms. The genetic testing result of the patient’s children was also positive for the RETC634R mutation. The similar mutation in exon 11 of the RET gene was identified in the patient’s two offsprings, II:1 and II:3 (Figure 1). Hence, these children are being monitored carefully as pre-symptomatic MEN2A cases.
**DISCUSSION**

MEN2A is an inherited, autosomal dominant disorder that is caused by alterations in the RET proto-oncogene. In this case, the patient I:1 was identified with pheochromocytoma and medullary thyroid carcinoma. The genetic analysis revealed a causative mutation of MTC with a substitution from cysteine to arginine at codon 634 in exon 11 of the RET proto-oncogene. Clear associations have been documented between specific RET mutations (genotype) and the age of onset and aggressiveness of MTC as well as the presence or absence of other endocrine neoplasms (phenotype), such as pheochromocytoma or hyperparathyroidism (Table 1) (Machens et al., 2003). The genotype-phenotype correlation of the cysteine at codon 634 mutations with MEN2A associates with the highest penetrance of pheochromocytoma, which increases with age (25% by the age of 30 years, 52% by the age of 50 years, and 88% by the age of 77 years) (Imai et al., 2013). Additionally, cutaneous lichen amyloidosis is a rare disorder that occurs almost exclusively in MEN2A patients with the RET codon 634 mutation (Wells Jr et al., 2015).

Some overlaps exist between RET mutations and the resulting clinical subtype of MEN2. Approximately 98% of families with MEN2A have a RET mutation in exon 10 or 11. Mutations in the cysteine at codon 634 occur in about 87% of the families with MEN2A; and an identifiable RET mutation occurs in about 95% of the families with FMTC (Raue, Frank-Raue, 2012).
Mutation of the extracellular cysteine at codon 634 in exon 11 causes ligand-independent dimerization of receptor molecules, enhancing phosphorylation of intracellular substrates and cell transformation due to aberrant homodimerization (Chappuis-Flament et al., 1998). The transforming capacity of the c-RET examined in transfected NIH-3T3 cells has been shown to be dependent on specific mutated codons, with the C634R (TGC→CGC) mutant showing a 3 to 5 fold higher transforming activity compared with any exon 10 Cys mutants (Ito et al., 1997). Although the three-dimensional structure of the RET extracellular domain is still unknown, these cysteines likely form intramolecular disulfide bonds in the wild-type receptor while an unpaired cysteine appears in mutant one forming an activating intermolecular bridge (Santoro et al., 2002). Differences in dimerization induction intensities are a reasonable explanation for the phenotypes resulting from mutations of the different cysteines. In fact, the international RET mutation consortium analysis studied 477 MEN2 families from 18 tertiary referral centers, which did not include any kindred from Brazil, and demonstrated that specifically mutated RET codons correlated with MEN2 variants (Mulligan et al., 1993). Based on the finding of a significant association between the C634R mutation and MEN2A syndrome, specific changes in cysteine substitution at codon 634 are predicted to be able to affect the natural history of disease in MEN2A.

Table 1. Management of patients with different RET mutations (American Thyroid Association Guidelines Task et al., 2009).

| Characteristic/management | Codons 321,515,533,600,603,606,635,649,666,768,776,790,791,804,819,833,844,861,891,912 | Codons 609,611,618,620,630,631 | Condon 634 | Condon 918,883 |
|---------------------------|------------------------------------------------------------------------------------------------|---------------------------------|-------------|----------------|
| ATA risk level (2009)*    | A                                                                                             | B                               | C           | D              |
| MEN2 subtype              | FMTC                                                                                         | FMTC/MEN2A                      | MEN2A       | MEN2B          |
| MTC aggressiveness        | Moderate                                                                                      | High                            | Higher      | Highest        |
| MTC age of onset          | Adults                                                                                        | 5 years                         | Before the age of 5 years | First year of life |
| Timing of prophylactic thyroidectomy | When calcitonin rises/age 5 or 10 years                                                | 5 years                         | Before the age of 5 years | First months of life |
| Screening for Pheo         | Start at 20 years, periodically                                                              | Start at 20 years, annually     | Start at 8 years, annually | Start at 8 years, annually |
| Screening for HPT          | Start at 20 years, periodically                                                              | Start at 20 years, periodically | Start at 8 years, annually | Start at 8 years, annually |

ATA: American Thyroid Association. FMTC: familial medullary thyroid carcinoma. HPT: Hyperparathyroidism. MTC: medullary thyroid carcinoma. Pheo: Pheochromocytoma.

*Risk for aggressive MTC; level D is highest risk

Approximately 98% of all mutations associated with MEN2A are influenced by pathogenic RET variants, which affect the cysteine codons of the extracellular domain (codons 609, 611, 618, 620, 630, and 634) (Eng et al., 1996; Mulligan Lois et al., 1993). Such mutations with disrupted cysteine may allow the partner cysteine to become available for aberrant disulfide bonding with other mutant RET molecules. The activation of constitutive receptor dimerization induced by aberrant disulfide bonding leads to overregulation of the...
extracellular signals for processes as diverse as differentiation, cell growth, survival, and programmed cell death (Santoro et al., 1995). On the basis of RET codon mutations, current American Thyroid Association (ATA) risk categories for MTC are subdivided as follows: (i) Highest risk: patients with MEN2B and RET codon M918T mutation; (ii) High risk: patients with RET codon C634 (C634F/G/R/S/W/Y) and A883F mutations; (iii) Moderate risk: patients with MTC and RET codon mutations other than RET codon C634 and M918T (Wells Jr et al., 2015) and the recommendations for the family members who genetically tested positive are prophylactic thyroidectomy before the age 5, annual albumin-corrected calcium or ionized serum calcium measurements (with or without serum intact parathyroid hormone) beginning at the age of 8, and annual plasma free metanephrines and normetanephrines or 24-hour urine collection for metanephrine and normetanephrine beginning by the age 20 (Pop et al., 2012). In this particular case, the replacement of a cysteine 634 residue with arginine was detected not only in the patient, but also in his daughter and son. This result indicated that the family carried an inherited type of MTC. However, different family members carrying the same RET variation can display different levels of MTC. This might result from diverging levels of genetic penetrance or the presence of genetic modifiers (Pop et al., 2012).

The association between disease phenotype and RET genotype may also have important implications for the management of clinical presentations other than MTC in MEN2 patients and their families. If the genotype fully correlates with certain phenotypic features, then, basing on the patient's genotypes, a clinician can decide whether intense screening for pheochromocytoma or hyperparathyroidism is necessary in those patients with mutations associated with a higher risk of disease. For such patients, pheochromocytoma should be removed before a thyroideectomy is performed, since an undetected pheochromocytoma could be a reason for an intraoperative fatal or a hypertensive crisis. Prophylactic thyroidectomy is believed to be an effective treatment for RET mutation carriers. Recommendations for the time point of a prophylactic thyroidectomy are based on the patient's genotype-phenotype correlations (Raue and Frank-Raue, 2012). Annual serum calcitonin screening should begin for children with MEN2A at age 3–5 years. However, caution should be exercised when interpreting calcitonin results for children under 3 years of age, especially children under 6 months of age (American Thyroid Association Guidelines Task et al., 2009).

Due to MTC being malignant, the prognosis of patients with MEN2A was closely related to the early detection and treatment of MTC. Molecular diagnostic testing in which RET genetic screening is the key tool has been the mainstay of the MTC clinical management for many years. Moreover, an important benefit is a comprehensive analysis of molecular alterations in MTC that allows rapid identification of mutations. The acceleration of this process allows for patients to be quickly classified into different risk levels (Wells Jr et al., 2015), providing them with tailored therapeutics for each specific case. Patients detected by screening have shown to have improved survival as compared to the index patients and patients with sporadic disease (Abraham et al., 2011). This underlines the importance of screening for RET mutations and initiating treatment at an early stage to improve outcomes and survival. Identifying mutations in asymptomatic pathogens adds value to the existing data from Vietnam, especially since there is a paucity of information in this area.

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XÉT NGHIỆM ĐI TRUYỀN XÁC ĐỊNH NGUY CO TIỂM ÁN CỦA BỆNH ĐA U NỘI TIÉT TRONG MỘT GIA ĐÌNH Ở VIỆT NAM

Nguyễn Hải Hà1,2, Nguyễn Thị Thanh Hoa1, Phạm Thị Dung3, Nguyễn Huy Bình4, Nguyễn Đăng Tôn1,2

1Viện Nghiên cứu hệ gen, Viện Hàn lâm Khoa học và Công nghệ Việt Nam
2Học viện Khoa học và Công nghệ, Viện Hàn lâm Khoa học và Công nghệ Việt Nam
3Bệnh viện E, Hà Nội, Việt Nam
4Trường Đại học Y Hà Nội, Việt Nam

TÔM TÁT

Hội chứng đa u nội tiết type 2A (MEN2A) là một hội chứng di truyền hiềm gặp, được đặc trưng bởi ung thư biểu mô tuyến giáp thể tuyến (medullary thyroid carcinoma), u tủy thường thân (pheochromocytoma), u tuyến cận giáp (hyperparathyroidism) và đột quỵ la bệnh rối loạn chuyển hóa tinh bột ở da (lichen amyloidosis). Hội chứng MEN2A gây ra một dòt bệnh dòng máu trên gen tiến sinh ung thư (proto-oncogene) RET và được di truyền theo tính trạng trên nhiễm sắc thể thường. Trong nghiên cứu này, chúng tôi báo cáo một trường hợp hiềm gặp ở bệnh nhân nam 44 tuổi ở Việt Nam bị ung thư biểu mô tuyến giáp thể tuyến và u tủy thường thân. Kết quả xét nghiệm di truyền phát hiện có một thay thế nucleotide năm ở exon 11 của gen RET (c.1900T> C, p.Cys634Arg), đã được báo cáo là đột biến gây bệnh MEN2A. Các xét nghiệm di truyền ở các thành viên khác trong gia đình cho thấy đột biến này xuất hiện ở con gái (14 tuổi) và con trai (8 tuổi) của bệnh nhân. Mặc dù hai người con chưa có triệu chứng của bệnh, nhưng cần lưu ý về nguy cơ cao tiến triển bệnh do đột biến gen RET được di truyền từ bố. Do đột biến được xếp vào nhóm nguy cơ cao trong hướng dẫn về ung thư biểu mô tuyến giáp thể tuyến nên việc phát hiện đã gây ra sự lo ngại trong C634R, cũng như các chiến lược theo dõi nghiêm ngặt để giảm tỷ lệ mắc và tử vong.

Từ khoá: Da u nội tiết 2A, gen tiến ung thư RET, đột biến p.C634R