

Mutation analysis in two Chinese families with multiple endocrine neoplasia type 1

Análise de mutações em duas famílias chinesas com neoplasia endócrina múltipla tipo 1

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ABSTRACT

Objective: This study aimed at identifying mutations in two Chinese genealogies with MEN1. **Subjects and methods:** Three members of two Chinese families with MEN1 were enrolled in this study, and all of the coding regions and adjacent sequences of the *MEN1* gene were amplified and sequenced. **Results:** A recurrent mutation of heterozygous change T>A at IVS 4+1 was found in family I, and a novel insGAGGTGG mutation (c.703-709dup7bp) resulted in a frameshift (p.A237Gfsx13) in family II. **Conclusion:** We are able to add a new mutation of *MEN1* gene in Chinese patients with MEN1 that will be useful for the diagnosis and treatment of the disease. Arq Bras Endocrinol Metab. 2012;56(3):184-9

Keywords

Multiple endocrine neoplasia type 1; *MEN1* gene; germline mutation; menin

RESUMO

Objetivo: O objetivo deste estudo foi identificar as mutações em duas famílias chinesas com NEM1. **Sujeitos e métodos:** Três membros das duas famílias chinesas foram estudados. Em todos eles, as regiões codificadoras e sequências adjacentes do gene *MEN1* foram amplificadas e sequenciadas. **Resultados:** Uma alteração heterozigota recorrente de T>A em IVS 4+1 foi encontrada na família I, e uma nova mutação insGAGGTGG (c.703-709dup7bp) levou a um *frameshift* (p.A237Gfsx13) na família II. **Conclusão:** Adicionou-se uma nova mutação ao gene *MEN1* em pacientes chineses com diagnóstico de NEM1 que vai ser útil no diagnóstico e tratamento da doença. Arq Bras Endocrinol Metab. 2012;56(3):184-9

Descritores

Neoplasia endócrina múltipla tipo 1; gene *MEN1*; mutação germinal

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INTRODUCTION

Multiple endocrine neoplasia type 1 (MEN1), first reported by Wermer (1) in 1954, is an autosomal dominant disorder characterized by varying combinations of tumors involving the parathyroid (90%-100%), enteropancreatic neuroendocrine tissues (30%-75%) and the anterior pituitary (50%-65%), with penetrance higher than 90% by age 50 (2,3). Clinical manifestations of MEN1 are generally related to their products of secretion and, less frequently, to their primary sites or metastasis (4). In the absence of treatment, patients with MEN1 die earlier (5). Nonfunctional pancreatic

endocrine tumors have a much less favorable prognosis. Approximately 50% to 80% of these neoplasms recur or metastasize, and up to one third of patients already have metastases at the initial presentation (6). Nonfunctional pancreatic tumors frequently determine hepatic metastasis, while duodenal gastrinomas determine, in most cases, metastasis to local lymph nodes (5,7-9). Other tumors with lower penetrance in MEN1 are malignant and frequently diagnosed later on. These tumors are: neuroendocrine thymic and bronchial tumors (10), glucagonomas (11), somatostatinomas (12), and vipomas (13). The gene causing MEN1 is

localized to chromosome 11q13. The *MEN1* gene consists of 10 exons that span approximately 9 kb of genomic DNA. *MEN1* germline mutations can be identified in more than 85% of MEN1 families.

In the present study, we analyzed and identified *MEN1* germline mutations in two Chinese families, supporting the practical importance of early detection and individualized surgical therapy for MEN1 patients.

SUBJECTS AND METHODS

Clinical data

Diagnosis of MEN1 was based on the presence of tumors in two or more of the three principal systems, i.e., parathyroid, anterior pituitary, and enteropancreatic neuroendocrine tissues (14). Detailed family histories were obtained from the probands and available family members. Individual tumors were classified according to clinical features.

Patient 1 of family I visited our hospital due to repeated hypoglycemic attacks and, at age 48, she received a hypophysectomy at another hospital. She had a history of hypertension, but no definite history of hereditary disease (Figure 1). Laboratory findings for the patient upon admission are summarized in table 1. Elevated levels of serum Ca²⁺, PTH, insulin, and gastrin, and decreased levels of blood glucose were noted. Parathyroid MIBI imaging showed right parathyroid with hyperparathyroidism (Figure 2A). CT scan of the pancreas revealed multiple nodules with partial calcification, as well as a left adrenal nodule (adenoma; Figure 2B-F). MRI images of the skull and brain found an irregular pituitary with homogeneous signal intensity. According to these findings, the patient was then diagnosed with MEN1 (pancreatic tumor, hyperparathyroidism with right parathyroid tumor, left adrenal adenoma). The right parathyroid tumor was completely removed by surgery, and pathology confirmed the diagnosis of adenoma (Figure 3A). During laparotomy, ultrasonography showed one nodule in the pancreatic head (diameter: 2.5 cm), and one nodule in the pancreatic tail (diameter: 1.5 cm). Subsequently, these two pancreatic tumors were resected. Pathology and immunohistochemistry confirmed the diagnosis of insulinomas for two pancreatic tumors (Figure 3B-E). No treatment for left adrenal adenoma was determined at this time, but the patient underwent regular follow-up.

Table 1. Laboratory findings in the two patients

	Reference range	Patient 1	Patient 2
Plasma glucose (mmol/L)	3.6 ~ 6.1	1.7	4.5
Insulin (μIU/mL)	2.0 ~ 16	47.58	9.8
Ca (mmol/L)	2.13 ~ 2.70	2.99	2.81
P (mmol/L)	0.81 ~ 1.45	0.68	0.75
PTH (ng/L)	11 ~ 62	596	521.7
PRL (ng/mL)	2.1 ~ 11.7	7.93	47.6
TSH (μIU/mL)	0.38 ~ 4.43	0.58	2.89
Gastrin (pg/mL)	< 100	180.7	48.9
GH (ng/mL)	< 2	0.1	2.3
ACTH (pg/mL)	0 ~ 45	6.4	25.0

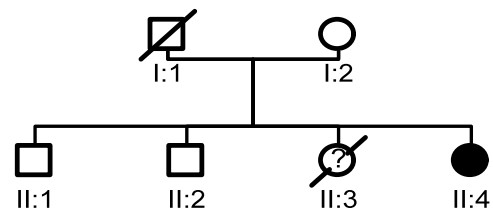


Figure 1. Genealogy chart of family I.

Patient 2 of family II visited our hospital due to recurrent diarrhea for about 4 years. At age 32, he received a hypophysectomy and postoperative radiotherapy at another hospital. His elder brother had undergone enucleation of a pancreatic insulinoma, and resection of a parathyroid adenoma (Figure 4). Laboratory findings for the patient upon admission are summarized in table 1. Elevated levels of serum Ca²⁺, PTH, PRL, and GH, and decreased serum phosphorous were found. Parathyroid MIBI imaging showed left parathyroid with hyperparathyroidism (Figure 5A). A nodule in the pancreatic head (size: 1.1 cm x 1.3 cm x 1.0 cm) and a nodule in the pancreatic tail (size: 1.3 cm x 3.4 cm x 2.9 cm) were found in the CT scan (Figure 5B-F). Endoscopy found a duodenal ulcer. Given these findings, the patient was diagnosed with MEN1. Left parathyroid tumor resection was performed, and the pathology showed that the parathyroid tumor was an adenoma (6.0 cm x 2.0 cm; Figure 6A). Pancreatic head and tail tumors were removed by surgery, and pathology and immunohistochemistry showed well differentiated neuroendocrine tumors (Figure 6B-K).

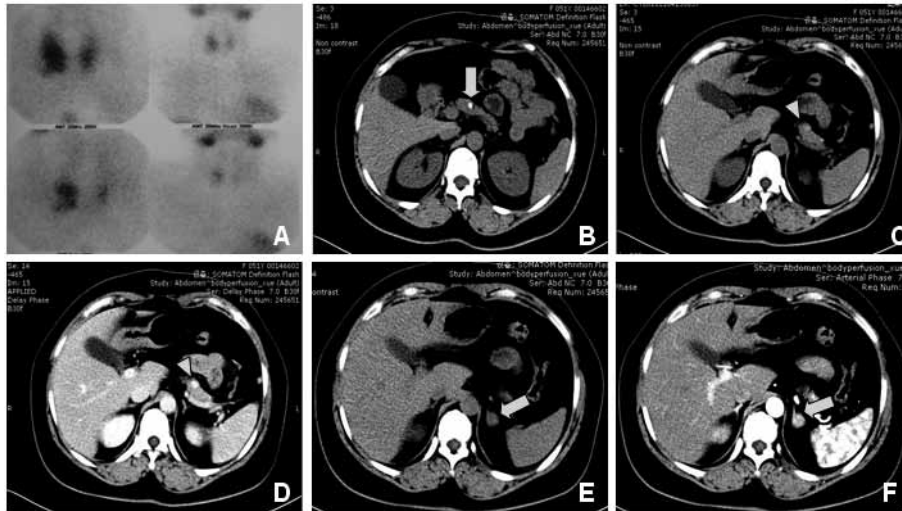


Figure 2. Imaging data of patient 1. ^{99m}Tc-MIBI scan (A) showed enlargement of a right parathyroid gland. CT scan showed nodule in pancreatic head with partial calcification (B), nodule in pancreatic tail (C, D), and nodule in left adrenal (E, F).

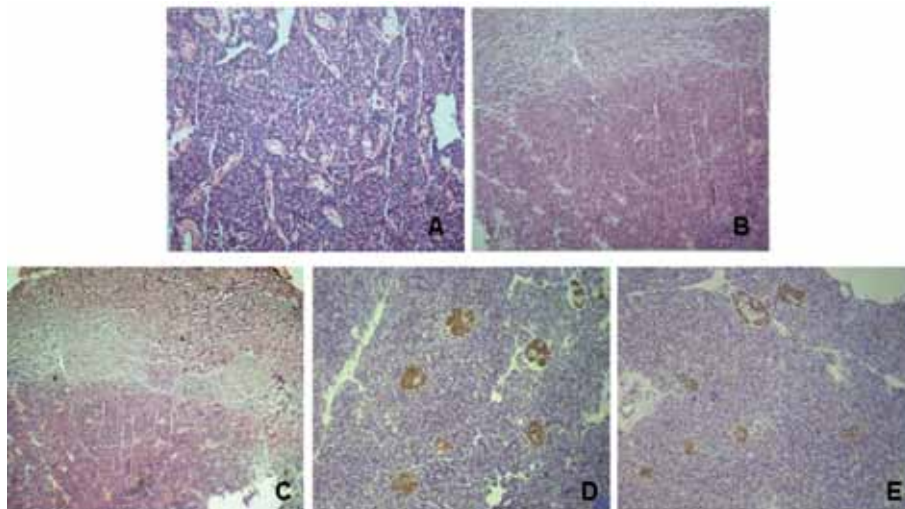


Figure 3. Histopathological section A shows parathyroid adenoma (HE, original magnification 100x), and sections B, C show an insulinoma in the pancreatic head and tail, respectively (HE, original magnification 100x). Immunohistochemistry sections D, E show positive immunohistochemical analysis for insulin of pancreatic head and tail tumor, respectively (original magnification 100x).

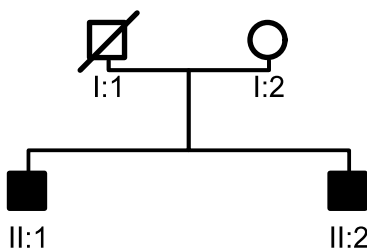


Figure 4. Genealogy chart of family II.

These two patients were diagnosed with MEN1 with all the characteristics of gastrointestinal neuroendocrine tumors, parathyroid adenomas, and pituitary tumors. The elder brother of the patient in family II also had history of insulinoma and parathyroid adenoma.

Participants were informed on the implications and purpose of this genetic study. A written informed consent was obtained from all patients that wanted to participate.

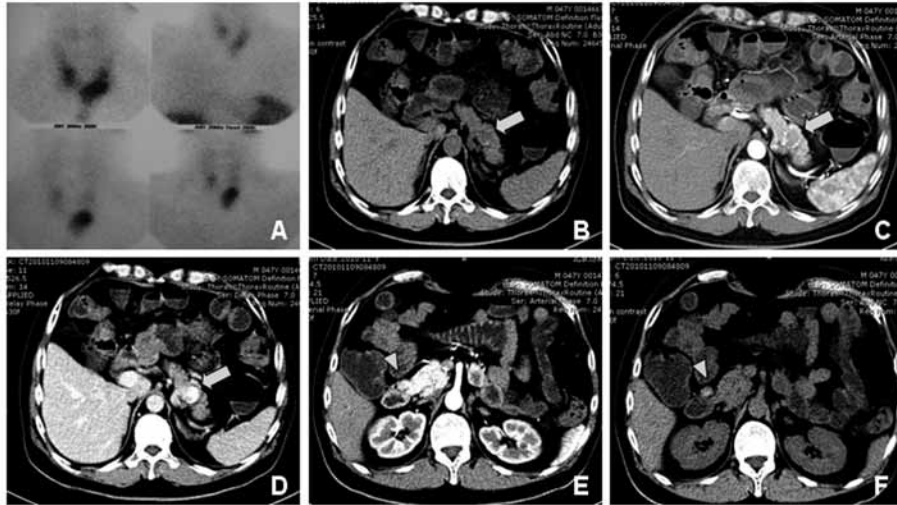


Figure 5. Imaging data of patient 2. ^{99m}Tc -MIBI scan (A) showed enlargement of left parathyroid gland. CT scan showed nodule in the pancreatic tail (B, C, D) and a nodule in the pancreatic head (E, F).

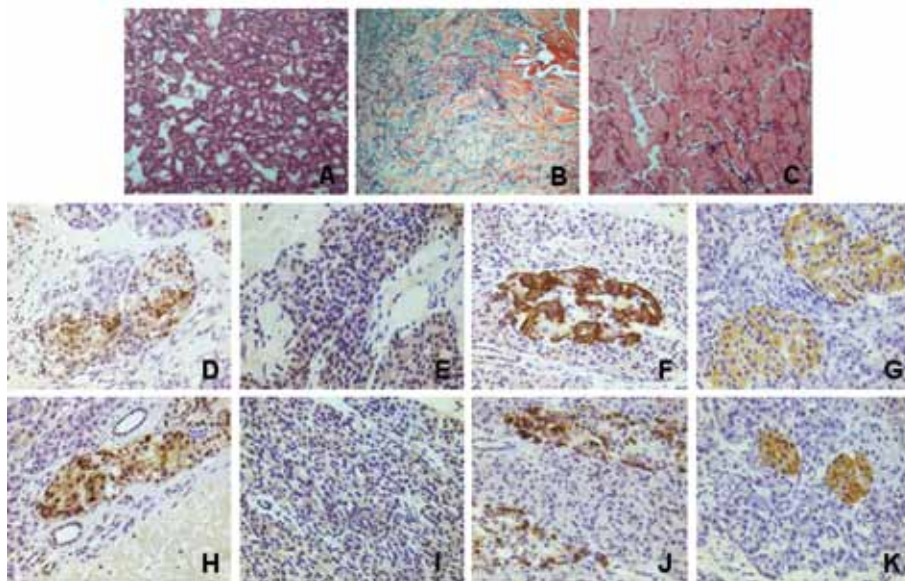


Figure 6. Histopathological section A shows parathyroid adenoma (HE, original magnification, 100x), sections B, C show well differentiated neuroendocrine tumors in the pancreatic head and tail, respectively (HE, original magnification, 100x). Immunohistochemistry sections D, E, F, G show positive immunohistochemical analysis of pancreatic head tumor for somatostatin, gastrin, glucagon, and insulin, respectively (original magnification, 400x), sections H, I, J, K show positive immunohistochemical analysis of pancreatic tail tumor for somatostatin, gastrin, glucagon, and insulin, respectively (original magnification, 400x).

DNA extraction and mutation screening

Venous blood samples were obtained from three patients of two families (1 female and 2 males) for genetic analyses. Genomic DNA was isolated from peripheral blood. Exons were amplified by polymerase chain reaction (PCR) (15). Purified PCR products were then sequenced.

RESULTS

By direct sequencing of *MEN1* coding region, a recurrent mutation of heterozygous change T>A at IVS 4+1 was found in family I (Figure 7). A novel insGAG-GTGG mutation (c.703-709dup7bp) resulted in a frameshift (p.A237Gfsx13) in family II (Figure 8).

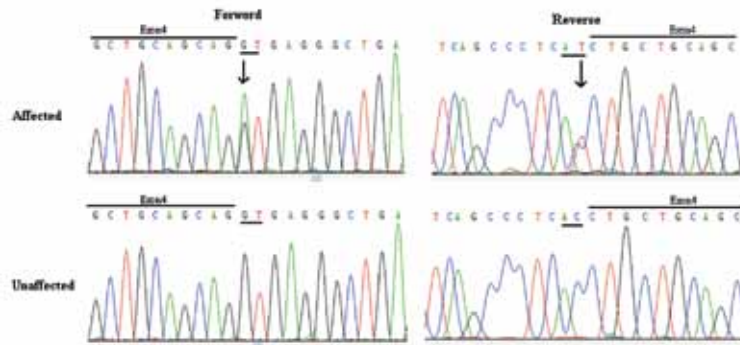


Figure 7. DNA sequence analysis of *MEN1* in unaffected and affected individuals of family I. A heterozygous change T > A at IVS4+1 was noted in affected individuals.

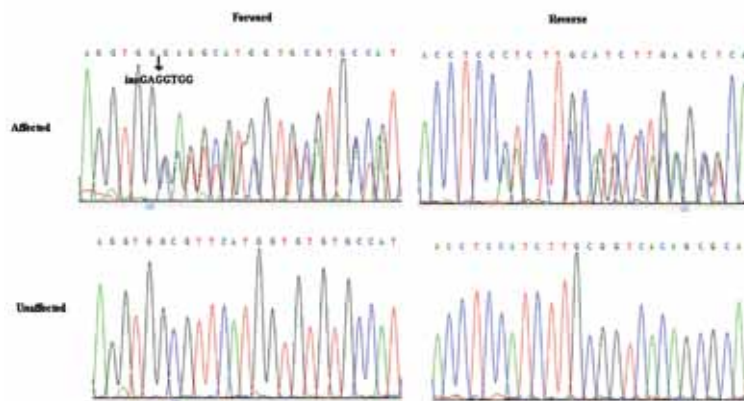


Figure 8. DNA sequence analysis of *MEN1* in unaffected and affected individuals in family II. An insGAGGTGG mutation (c.703-709dup7bp) was found, resulting in a frameshift (p.A237Gfsx13).

DISCUSSION

A total of 1,336 mutations of *MEN1* have been reported in the first decade following identification of this gene. The 1,133 germline mutations are scattered throughout the entire 1,830-bp coding region and splice sites of the *MEN1* gene, and consist of 23% nonsense mutation, 9% splice site mutations, 41% frameshift deletions or insertions, 6% in-frame deletions or insertions, 20% missense mutation, and 1% whole or partial gene deletions (16,17). The *MEN1* gene encodes a 610-amino acid protein referred to as menin, which is ubiquitously expressed, and is predominantly a nuclear protein in nondividing cells (18). However, in dividing cells, it is mainly found in the cytoplasm (19). Menin has at least three nuclear localization signals (20).

MEN1 germline mutations were identified in more than 85% of the *MEN1* families investigated when full-length sequencing of the open reading frame was performed (21-23). Until now, some genes were recently

identified as probable causes of *MEN1*-like condition, such as mixed lineage leukemia (*MLL*): *p15*, *p18*, *p21* and *p27* (24-27). However, the great diversity, together with widely scattered locations of the *MEN1* mutation and a lack of genotype-phenotype correlation, make such mutational screening time consuming, laborious, and expensive (28,29). Nevertheless, an integrated program of both mutational analysis, to identify mutant gene carriers, and biochemical screening, to detect the development of tumors, is advantageous and used by many centers (14,17,30). Thus, a DNA test identifying an individual as a mutant gene carrier is unlikely to lead to immediate medical or surgical treatment, but to earlier and more frequent biochemical and radiologic screening, whereas a DNA result indicating that an individual is not at risk will lead to no further clinical investigation. The identification of *MEN1* mutations may be of help in the clinical management of patients with this disorder and their families (17). Medications for

MEN1 should control most features of excess of some hormones (gastrin, PRL etc.). Surgery should control features of excess of other hormones (PTH and insulin). However, surgery has not been shown to prevent or cure MEN1-related cancers (14).

In conclusion, we have identified a novel insGA-GGTGG mutation (c.703-709dup7bp) resulting in a frameshift (p.A237Gfsx13) in a Chinese family. This finding extends our knowledge of the variety of genetic abnormalities associated with familial MEN1. Functional studies are necessary to evaluate and understand the impact of this insertion mutation on the clinical picture.

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