

A Germline Mutation in a Thai Family with Familial Multiple Endocrine Neoplasia Type 1

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Multiple endocrine neoplasia type 1, caused by the mutation in the *MEN1* gene, is an autosomal dominant disorder with over 95% penetrance characterized by hyperparathyroidism, pancreatic endocrine tumor and pituitary tumor. The authors performed a molecular analysis to identify a mutation in a Thai man with *MEN1*. He was found to be heterozygous for IVS6 + 1G to A. Two of his three children were also found to carry this mutation. The newly available genetic test for patients with *MEN1* in Thailand makes it possible to accurately DNA-based diagnose clinically suspected individuals and their presymptomatic members, which has important therapeutic impacts on them.

Keywords: Multiple endocrine neoplasia type 1, *MEN1* gene, Mutation analysis

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Multiple endocrine neoplasia type 1 (*MEN1*; OMIM 131100) is characterized by the combined occurrence of tumors of the parathyroid glands, endopancreatic neuroendocrine tissues and anterior pituitary. Less frequently, adrenal cortical tumors, carcinoid tumors, spinal ependymomas and lipomas can be present. Familial *MEN1* is similarly defined as at least one of the first degree relative of the index case having one of those three tumors⁽¹⁾. The *MEN1* gene, tumor-suppressor gene, is located on chromosome 11q13 and consists of 10 exons with 1830 bp, encoding a 610-amino-acid protein, menin⁽²⁾. The authors report a mutation of the *MEN1* gene at IVS6 + 1G to A in a Thai kindred affected by *MEN1*. To the authors' knowledge, this is the first published genetic analysis of *MEN1* in Thailand.

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Material and Method

Patient

The proband (Fig. 1), a 44-year-old Thai male, was admitted to the Department of Surgery of King Chulalongkorn Memorial Hospital due to incidentally finding a pancreatic and left adrenal mass from non-specific abdominal pain. The physical examination was normal except for a mass, 4x3 cm in size, at his thyroid bed. Markedly elevated serum calcium level (15.3;

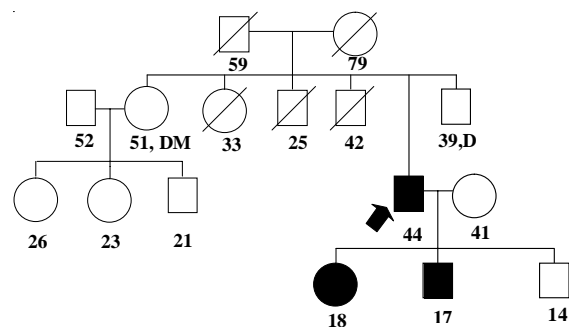


Fig. 1 Pedigree of the proband

normal range, 8.1-10.4 mg/dL) with suppressed serum phosphate level (1.7; normal range, 2.7-4.5 mg/dL) and the serum intact parathyroid hormone (PTH) level 1,202 (10-65 pg/mL) were compatible with primary hyperparathyroidism. The other laboratory and hormonal data were presented in Table 1 and 2, respectively.

Neck ultrasound discovered a mass sized 4.5x2.8 cm at his right thyroid bed with posterior extension indicating a parathyroid tumor, confirmed with MIBI scan (Fig. 2). A pituitary CT scan was normal. Distal pancreatectomy with left adrenalectomy was done. Subsequently, neck exploration and total parathyroidectomy was performed.

Table 1. Laboratory data on admission

Peripheral blood	
White blood cells	8,540/ μ l (4,500-11,000)
Red blood cells	4.97 x 10 ⁶ / μ l (4.2-6.2 x 10 ⁶)
Hemoglobin	14.5 g/dL (13-18)
Hematocrit	44.6% (42-54)
Platelet	424 x 10 ³ / μ l (150-450 x 10 ³)
Blood chemistry	
Plasma glucose	100 mg/dL (60-100)
Blood urea nitrogen	9 mg/dL (10-20)
Creatinine	0.9 mg/dL (0.5-2.0)
Total protein	7.1 g/dL (6.6-8.7)
Albumin	4.9 g/dL (3.5-5.0)
Globulin	2.2 g/dL (2.0-4.0)
Total bilirubin	0.83 mg/dL (0-1.0)
Direct bilirubin	0.05 mg/dL (0-0.2)
Aspartate aminotransferase	16 U/L (0-38)
Alanine aminotransferase	19 U/L (0-38)
Alkaline phosphatase	42 U/L (39-117)
Amylase	55 U/L (28-100)
Sodium	136 mmol/L (135-145)
Potassium	4.6 mmol/L (3.5-5.5)
Chloride	106 mmol/L (95-105)
Carbon dioxide	23 mmol/L (22-32)

Table 2. Hormonal data on admission

Free T ₄	1.2 ng/dL (0.8-1.8)
TSH	1.1 μ U/mL (0.3-4.1)
Prolactin	13.8 ng/ml (2-25)
IGF-I	122 ng/mL (50-475)
Urine metanephrine	78 μ g/24 hr (52-341)
Urine normetanephrine	182 μ g/24 hr (88-444)
Urine free cortisol	42 μ g/24 hr (39-498)
Serum morning cortisol	12 μ g% (7-25)
Gastrin	79.5 ng/L (44-104)

TSH: thyroid-stimulating hormone
IGF-I: insulin-like growth factor I

DNA sequence analysis

Informed consent was obtained from the patient and his children for research purposes. Genomic DNA was extracted from peripheral blood leukocytes by the phenol-chloroform method. The entire coding sequence of the *MEN1* gene was amplified by PCR as using primers and conditions as previously described⁽³⁾. PCR products were purified by ExoSAP-IT (USB, Ohio, USA) at 37°C for 15 min and 80°C for 15 min and sent for sequencing at Macrogen Inc, Seoul, South Korea.

Confirmation of the mutation by restriction fragment length polymorphism (RFLP)

For RFLP analysis, a 1417 bp PCR fragment of exon 3-6 was amplified, using forward and reverse primers indicated as previously described, from the

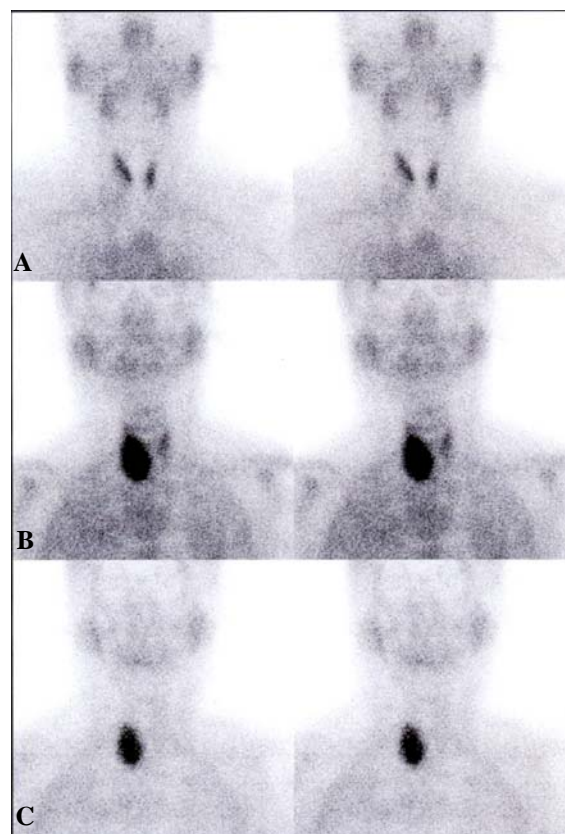


Fig. 2 (A) ^{99m}Tc-pertechnetate thyroid scan; unremarkable both lobes. (B) Early ^{99m}Tc-MIBI image; an area of abnormal increased uptake at the right lower pole region with non-specific uptake in the thyroid gland. (C) Delayed ^{99m}Tc-MIBI image; delayed radiotracer washout at the area mentioned, indicating tumor i.e. parathyroid tumor

proband and his three children. Aliquots (5 µl) of purified PCR samples were incubated overnight at 37°C with 0.1 µl of the restriction enzyme *DraIII* (Biolabs, New England, USA), 1 µl of NEB buffer, 0.1 µl of BSA and 3.8 µl of H₂O. An adequate amount was separated in 2% agarose gel and visualized with ethidium bromide.

Results

Histological findings

Neuroendocrine tumor of the pancreas (Fig. 3A), adrenal adenoma (Fig. 3B) and parathyroid adenoma (Fig. 3C) were confirmed by the histological

findings. The immunohistochemical staining of pancreatic tissues revealed positive immunoreactivity to synaptophysin, pancreatic polypeptide and chromogranin A but negative for vimentin, gastrin, glucagon and insulin.

Mutation analysis

The authors found a heterozygote G to A transition presenting in exon 6, at the donor splice site (Fig. 4A). This mutation created a new restriction site of *DraIII* causing a new band, 436 bp in the mutant sequence, which was found in the patient, his daughter and one of his sons (Fig. 4B).

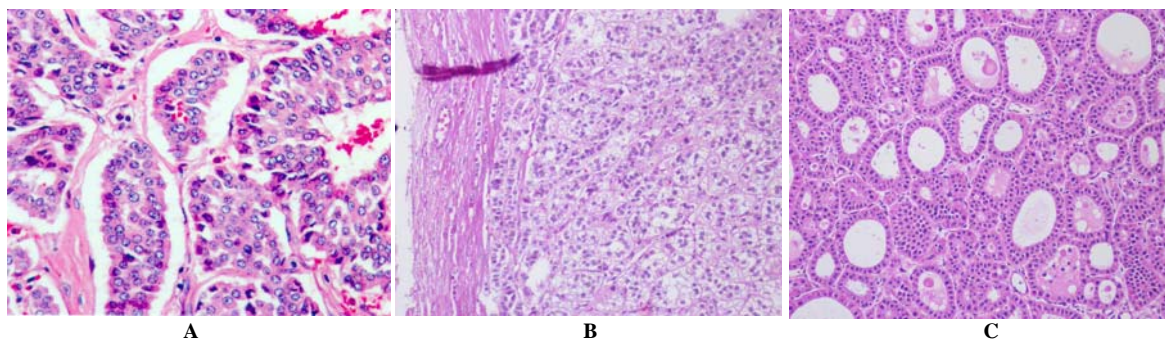


Fig. 3 (A): Histopathology of pancreas. Hematoxylin-and eosin-stained section showed tumor cells with speckle nuclei and a small to moderate amount of cytoplasm, arranging in cord, alveolar, and trabecular patterns, interspersed with thin vascular spaces. Mitoses were scattered. (B) Section of an adrenal tissue showed compacted alveolar clusters of cells contain abundant fine intracytoplasmic vacuoles. The adjacent non-neoplastic adrenal cortex reveals hyperplasia of zona glomerulosa (x100). (C) Sections from a parathyroid nodule showed varying size follicles with loose vascular stroma. Many of follicles contain eosinophilic proteinaceous material. The tumor composed predominantly almost entirely of oxyphil cells (x100)

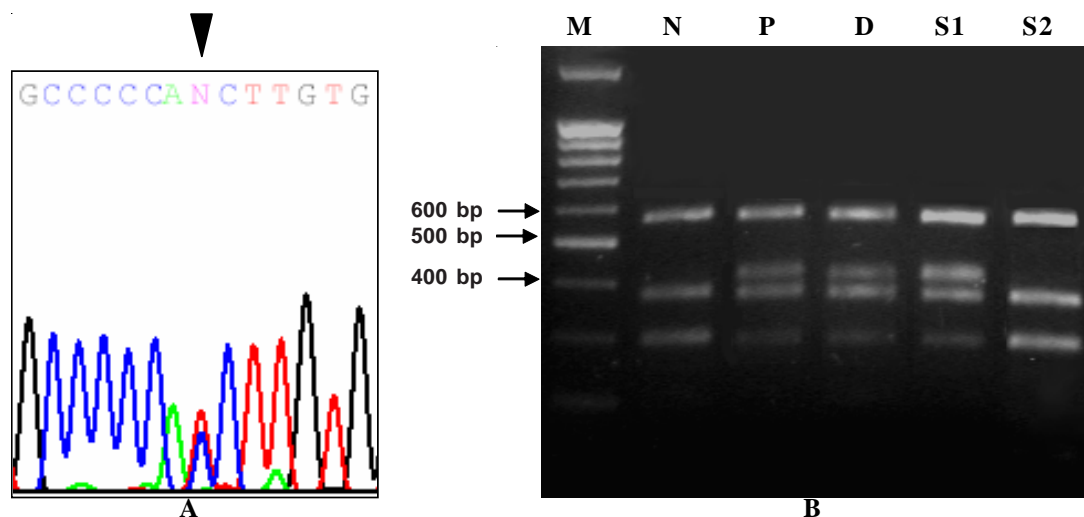


Fig. 4 (A) Reverse sequence of *MEN1* gene exon 6. The arrow head indicates mutation at IVS6 + 1 G > A. (B) Restriction enzyme *DRAIII* detected IVS6 + 1 G > A mutation in *MEN1* gene. Lane M represented a marker; Lane N served as a normal control; Lane P the proband; Lane D the proband's daughter; Lanes S1 and S2 the proband's sons. The 436 bp band in lane P, D and S1 indicated that the proband, his daughter and one of his sons were heterozygous for the IVS6 + 1G > A mutation

Discussion

The presented patient had a parathyroid adenoma, pancreatic endocrine tumor, and adrenal adenoma, which were typical manifestations of MEN1. This syndrome should be suspected in a patient presenting with two of the three main endocrine tumors (parathyroid gland, entero-pancreatic tissue, pituitary gland)⁽⁴⁾. Primary hyperparathyroidism is the most common endocrinopathy in MEN1 and usually expresses as the first clinical presentation. Total parathyroidectomy with a fresh graft parathyroid autograft to the forearm or intentional total parathyroidectomy with life-long treatment of vitamin D analog is recommended⁽¹⁾. The prevalence of entero-pancreatic islet tumors varies from 30-75% after the age of 40 yr⁽⁵⁾. Non-functioning type including pancreatic polypeptide is present in about one fifth of MEN1 patients. Adrenal tumor has been found in up to one third of patients and characterized as bilateral hyperplasia or adenoma, and occasionally even carcinoma⁽⁶⁾.

Genetic testing of the patient and his family members at risk for MEN syndrome is the crucial step to diagnosis and allows one to identify individuals carrying mutant alleles with a very high specificity and sensitivity. Analysis of the *MEN1* gene is now postulated to group 1 cancer predisposition testing by the American Society of Clinical Oncology (ASCO)⁽¹⁾. Germline mutation of the *MEN1* gene has been reported in more than 80% of familial types and about half of sporadic types^(3,7). There is no correlation between the mutation type and localization with respect to MEN1 phenotype⁽⁸⁾. The authors have described a *MEN1* gene mutation in exon 6 at the donor splice site, which was previously reported by Mutch et al⁽³⁾, causing a new splice acceptor site nine base-pairs 5' to exon 5. From the RFLP study the authors identified the presented patients' children carried the mutation who are also subjected to periodic screening for endocrine tumor-related MEN1. The age-related penetrance of all features is near zero below the age 5 years, rising to above 50% by 20 years, and above 95% by 40 years^(4,7). Biochemical screening such as serum calcium is recommended yearly, with tumor

imaging recommended less frequently (every 3-5 years) and should continue for life⁽¹⁾.

In conclusion, the present study shows the importance and usefulness of genetic diagnosis, which improved the quality of diagnosis and treatment in the presented patient and his family members with hereditary cancer syndrome.

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การกลายพันธุ์ในยีน MEN1 ของครอบครัวผู้ป่วยไทยที่เป็น Multiple endocrine neoplasia type 1

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Multiple endocrine neoplasia type 1 (MEN 1) เป็นโรคที่ถ่ายทอดพันธุกรรมแบบจีนเด่นบนออโตโซม มีลักษณะที่สำคัญคือ primary hyperparathyroidism, endocrine pancreatic tumor และ anterior pituitary tumor คณะผู้วิจัยได้พบการกลายพันธุ์ที่ตำแหน่ง IVS6+1G to A บนจีน MEN1 ในผู้ป่วยชายไทยที่มีอาการ และอาการแสดงเข้าได้กับกลุ่มโรค MEN1 นอกจากนี้ยังตรวจพบการกลายพันธุ์ดังกล่าวในบุตรสาวและบุตรชายของผู้ป่วยซึ่งยังไม่มีความผิดปกติทางคลินิก การตรวจการกลายพันธุ์ถือว่าเป็นวิธีที่มีความแม่นยำและสำคัญในการวินิจฉัยโรค MEN1 และยังสามารถใช้ในการค้นหาโรคนี้ในกลุ่มญาติของผู้ป่วยซึ่งอาจยังไม่มีความผิดปกติทางคลินิก เพื่อประโยชน์สำหรับการป้องกันและรักษา
