

# A novel germline mutation of the *MEN1* gene caused multiple endocrine neoplasia type 1 in a Chinese young man and 1 year follow-up

W. LIU, X. HAN, Z. HU, X. ZHANG, Y. CHEN<sup>1</sup>, Y. ZHAO<sup>2</sup>, L. JI

Department of Endocrinology and Metabolism, Peking University People's Hospital, Beijing, China

<sup>1</sup>Department of Pathology, Peking University People's Hospital, Beijing, China

<sup>2</sup>Department of Nuclear Medicine, Peking University People's Hospital, Beijing, China

**Abstract. – BACKGROUND:** Multiple endocrine neoplasia type 1 (MEN 1) is an autosomal dominant cancer predisposition syndrome which manifests a variety of endocrine and non-endocrine neoplasms and lesions. Because of its complexity in clinical manifestations, it is always difficult to set up the diagnosis in the early stage of the disease.

**AIM:** Using genetic diagnosis to identify and describe the process of the disease from the very beginning and followed the treatment result in 1 year.

**MATERIALS AND METHODS:** In this assay, a Chinese young man aged 31 with parathyroid hyperplasia, suspected gastrinoma and an enlarged pituitary with elevated level of prolactin (PRL) and growth hormone (GH) was admitted to our Department ward. We performed genetic analysis in his family and described a new nonsense mutation at codon 308 in exon 6 of the *MEN1* gene, where a cytosine residue was exchanged for guanine residue (TCA > TGA), and a termination codon (S308X) occurred. During the 1 year follow up, typical manifestations emerged in this kindred and further confirmed the diagnosis of familial MEN 1.

**CONCLUSIONS:** We presented a case of MEN 1 from its early stage and followed the progression. Meanwhile, the mutation in this kindred has not been reported and our finding can contribute to better understanding about this disease.

*Key Words:*

Multiple endocrine neoplasia type 1, MEN 1, Nonsense mutation, *MEN1* gene.

## Introduction

Multiple endocrine neoplasia type 1 (MEN 1) is inherited as an autosomal dominant tumor syndrome characterized by development of tumors

in several endocrine or nonendocrine organs. MEN 1 is a rare disease with an estimated prevalence of 10/100,000<sup>1</sup>. And this disease has a high degree of penetrance such that more than 99% of mutation carriers develop endocrine tumors and clinical manifestations by the fifth decade<sup>2</sup>. The practical clinical definition of MEN 1 is a case with 2 of the 3 main MEN 1 related endocrine tumors (parathyroid tumor, entero-pancreatic endocrine tumor, and pituitary tumor). However, the clinical symptoms of MEN 1 might be complex because not only these three typical organs are involved, the adrenal tumor, lipoma, collagenoma and plenty other types of neuroendocrine tumor might also be part of the disease.

The *MEN1* gene is located on chromosome 11q13 and encodes a 610-amino acid protein menin. Menin acts as a tumor suppressor by binding directly or indirectly to the proteins regulating transcription, DNA processing, or DNA repair. A loss of function mutation can lead to defective menin protein function resulting in unregulated cell growth and tumor formation, following Knudson's two-hit hypothesis. Therefore, *MEN1* is believed to be a tumor suppressor gene. Up to today, nearly 500 germline mutations spreading across all the coding sequences of *MEN1* gene have been reported<sup>3</sup>. In all these mutations, approximately 15% are nonsense mutations, and missense mutations, frameshift deletions or insertions, splice site mutations, and in-frame deletions explain the rest of the mutations.

Here we report a case of MEN 1 from its early onset and follow the progression. Furthermore, we also show in this kindred a novel heterozygous C to G change in exon 6 of the *MEN1* gene resulting in replacement of the wild serine codon (TCA) with a termination codon (TGA) at position 308 of the resultant menin protein.

Corresponding Author: Xueyao Han, MD; e-mail: xueyaohan@sina.com;

Corresponding Author: Linong Ji, MD; e-mail: jiln@bjmu.edu.cn

## Materials and Methods

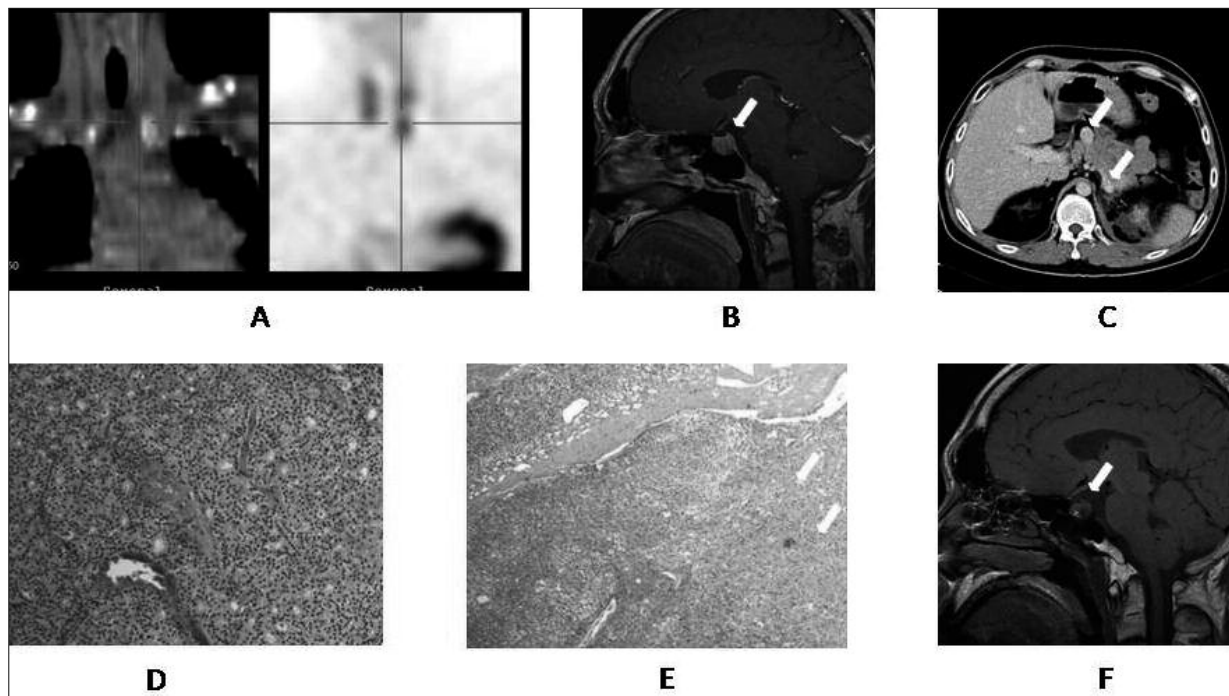
In December 2010, a 31-year-old Chinese young man (proband) with hyperglycemia and multiple stones in urinary tracts was admitted to our Department. He also complained of nausea, acid reflux, intermittent diarrhea, and reduced sexual function. No other remarkable family history was provided at that time.

All the hormones were tested by chemiluminescence. A written informed consent was obtained from all the participants in this study. This study was also approved by the Ethics Committee of Peking University People's Hospital.

Sestamibi single-photon emission computed tomography (SPECT) of the parathyroid gland showed abnormal uptake foci located in the lower pole of left lobe of the thyroid which suggesting parathyroid hyperplasia or adenoma (Figure 1A). An enlarged pituitary which was 10.6mm in height was detected on the magnetic resonance image (MRI), but no definite tumor was found even after contrast scan (Figure 1B). Computerized tomography (CT) scan of the urinary system detected a large (4×4 cm) tumor located in the

left adrenal gland. Abdominal CT scan was also examined and two tumors in the pancreas was discovered (Figure 1C). The laboratory findings of the patient upon admission were summarized in Table I. Elevated level of plasma glucose, serum calcium ( $\text{Ca}^+$ ), serum creatinine (Cr), parathyroid hormone (PTH), serum PRL (8AM), serum GH (8AM), serum insulin-like growth factor-I (IGF-I), serum gastrin, decreased level of serum potassium ( $\text{K}^+$ ), an elevated 24-h urine  $\text{Ca}^+$  and inappropriate increased 24-h urine  $\text{K}^+$  were noted. GH levels during the 75 g-oral glucose tolerance test (OGTT) were also measured<sup>4</sup>, and the results showed that the secretion of GH was not repressed by elevated level of plasma blood glucose (Table II). Cortisol and adrenocorticotrophic hormone (ACTH) rhythms did not show abnormal secretion. Plasma aldosterone to renin ratio ( $\text{pg/ml vs ng ml}^{-1} \text{ h}^{-1}$ ) was within the normal range. Meanwhile, the level of norepinephrine, adrenaline, and dopamine in both blood and urine were in the normal range.

According to these clinical findings above, the diagnosis of MEN 1 was highly suspected. On the third day after admission, the patient suf-



**Figure 1.** Abnormal imaging and pathology for the proband. **A**, SPECT of the parathyroids showed abnormal uptake foci located in the left. **B**, Pituitary MRI showed an enlarged pituitary (height: 10.8 mm). **C**, Abdominal SC scan showed 2 tumors in the pancreas. Abnormality was denoted by arrows. (4.1×3.9 cm). **D**, Pathology of resected parathyroid gland showed hyperplasia (HE stain, ×100). **E**, Abdominal CT scan showed two tumors in the pancreas. Abnormality was denoted by arrows. **F**, Pituitary MRI showed an reduced size than B (height: 9.6 mm).

**Table I.** Laboratory findings in the proband.

	The proband (upon admission)	The proband (12 month after diagnosis)	Reference range
Plasma Glucose (mmol/L)	6.31	5.52	3.3-6.1
Ca (mmol/L)	2.84	2.40	2.1-2.8
K (mmol/L)	2.84	3.62	3.5-5.5
Cr (μmol/L)	108	143	20-106
PTH (pg/ml)	113.0	39.5	15-88
24-h urine Ca (mmol/day)	13.16	–	2.5-7.5
24-h urine K (mmol/day)	44.64	–	25-100
PRL (ng/ml)	> 200.00	51.1	2.64-13.13
GH (ng/ml)	2.9	1.26	0.003-0.971
IGF-1 (ng/ml)	922	278	115-307
Gastrin (pg/mL)	176.9	202.3	0-100

ferred from watery diarrhea with up to 2-3 bowel movements per day and stool test revealed white blood cells 2 per high power field (HP) twice. Because infectious bowel disease can't be excluded, we added levofloxacin to the fluid infusion therapy. However, the patient's symptoms were not relieved, and he developed vomiting the next day. He vomited every 1-2 hours and each time the volume ranged from 300 to 500 mL. We stopped the antibiotic treatment and increased the total volume of infusion, and we also added famotidine considering elevated level of serum Ca<sup>+</sup> might induce acid reflux and vomiting. However, the patient still suffered from severe diarrhea and vomiting. His blood pressure even dropped to 90/60 mmHg because of rapid loss of body fluid. According to these clinical symptoms, the two tumors in the pancreas were suspected as gastrinoma or VIPoma, so we used omeprazole instead of famotidine. After that, the patient's symptoms relieved dramatically. On the next day, gastroscope was taken and showed regurgitant esophagitis and multiple gastric ulcers. Pathology result showed inflammation and no *Helicobacter pylori* was found. Since then, gastrinoma was highly suspected because proton pump inhibitors have no effects on VIPoma.

Since some of the pancreas tumors are nonfunctional and substantial proportion of gastrinomas lay in duodenum, we further ordered endoscopic ultrasound. But no lesion was found.

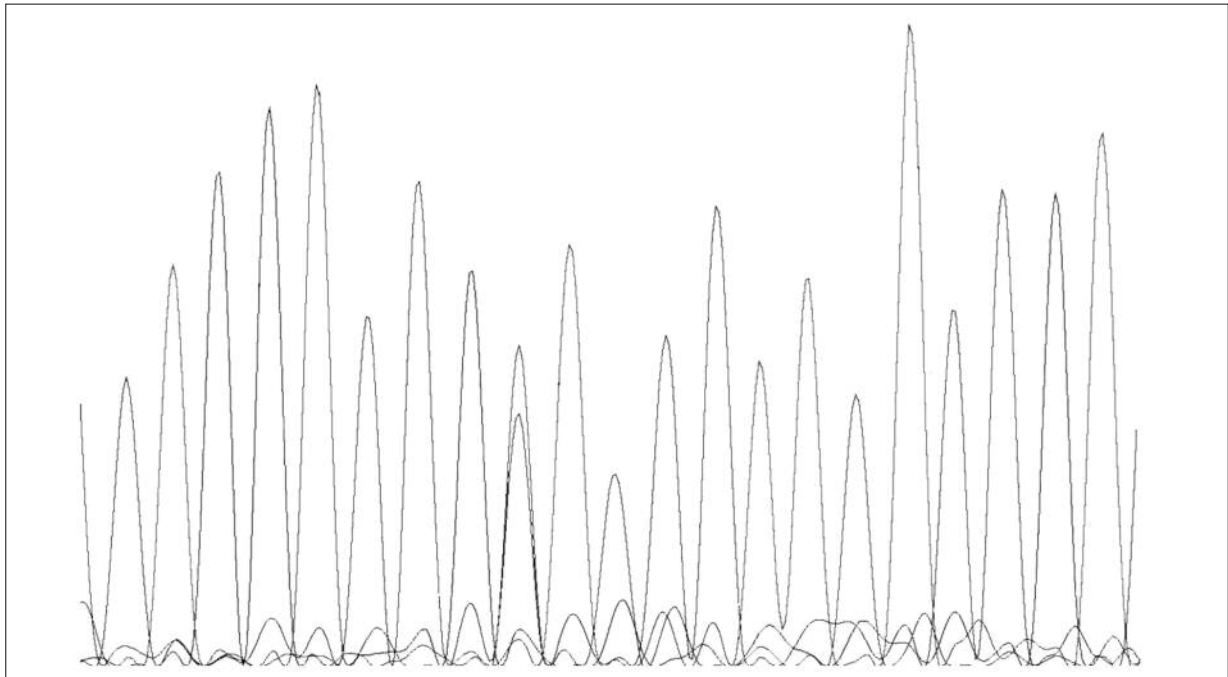
By then, the diagnosis of MEN 1 was established and we did further genetic research in this kindred. Blood samples were drawn from the patient, his parents and 2 siblings and DNA was extracted from peripheral blood leukocytes. The coding region of the *MEN1* gene and exon-intron boundaries were amplified by polymerase chain reaction (PCR) using Taq DNA polymerase. PCR primers and sequencing primers were designed according to published assay<sup>5</sup>. PCR products were then purified and sequenced by ABI 3130XL.

## Results

We identified a novel nonsense mutation (TCA TGA) at codon 308 in exon 6 of the *MEN1* gene (Figure 2), and that result in a serine to termination condon mutation (S308X). The mutation was confirmed by reverse sequencing and it was detected in the proband, his father and sister. This mutation was not detected in his mother, brother and 50 other healthy subjects.

**Table II.** OGTT-GH repressive test.

	Plasma glucose (mmol/L)		Insulin (μU/mL)		GH (ng/ml)	
	Upon admission	12 month later	Upon admission	12 month later	Upon admission	12 month later
0 min	6.3	4.9	38.2	14.8	4.0	1.5
30 min	12.9	11.1	400.8	235.0	3.7	1.2
60 min	17.9	8.8	536.5	217.0	3.5	1.3
120 min	8.9	4.3	698.8	44.8	3.0	1.5
180 min	2.8	3.5	84.6	12.0	3.6	4.6



**Figure 2.** Partial genetic analysis of exon 6 of the MEN1 gene. Analysis of germline DNA from the family members revealed a novel mutation (Ser313stop) in the patients of this family.

Then we used calcitonin to maintain his calcium level to normal range and kept on giving him omeprazole for the preparation of surgery. After 1 month, the patient had parathyroid surgery. During the procedure, three parathyroids were discovered and two of them located in lower pole of both lobes of the thyroid were enlarged. The enlarged glands were resected and pathology showed parathyroid hyperplasia (Figure 1D). After the operation, the serum PTH and  $\text{Ca}^+$  level returned to normal range without taking calcitonin. PRL and IGF-I remained elevated, so we added bromocriptine 2.5 mg per day.

2 month later, the patient went back to our Hospital to have open stone surgery and the adrenal mass was removed. The pathology result confirmed the diagnosis of adrenal adenoma (Figure 2E). After the surgery he kept on taking omeprazole and bromocriptine and still relied on potassium chloride to maintain the serum  $\text{K}^+$  level to the normal range.

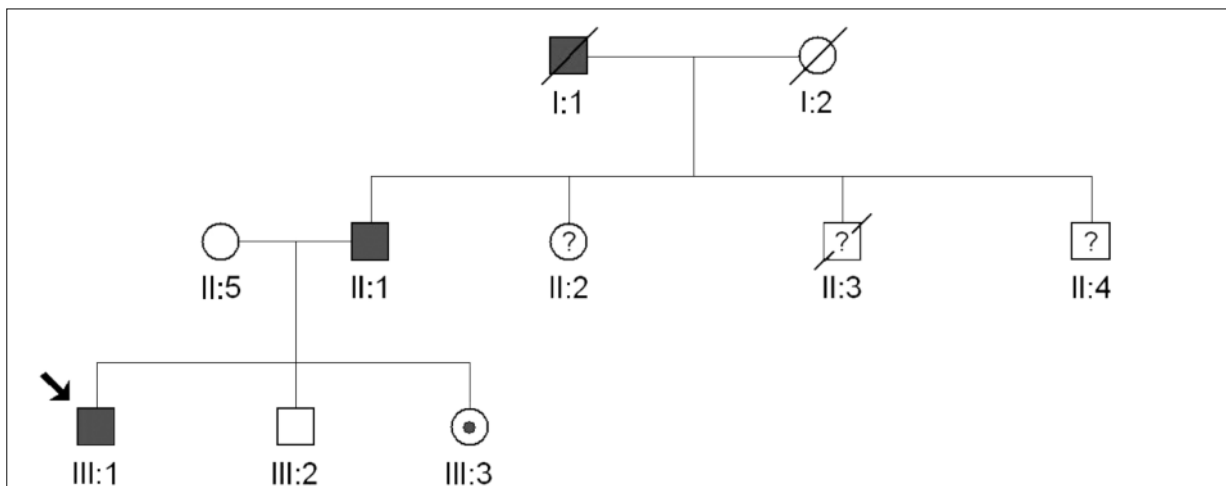
On the subsequent visit 3 months later, the patient informed us that his father was diagnosed with advanced lung cancer and he died in 1 month. His calcium level was also elevated but no exact result was provided. The patient recalled that his grandfather died at an early age without explicit reason (Figure 3). During the 3 months, the patient took omeprazole everyday and once with-

drawaled, diarrhea reappeared. He didn't took bromocriptine according to our prescription even though his sexual function was improved. The level of IGF-I is still high and gastrin grew even higher. CT scan showed no enlargement of the two pancreas tumors. The dosage of bromocriptine was doubled and we fully explained to the patient the importance of taking that.

Six month later, the patient's PRL and GH level all dropped significantly (see Table I), and the level of IGF-1 even dropped to normal range. GH levels during the OGTT were tested again and the results showed that the glucose metabolism state improved and GH levels decreased significantly (Table II). Pituitary MRI revealed that the size of the pituitary was decreased (Figure 1F).

## Discussion

In this essay, we presented a case of MEN 1 with parathyroid hyperplasia, multiple pancreatic tumors highly suspected gastrinoma, enlarged pituitary with elevated level of PRL and GH/IGF-I. Considering the patient's father who carried the same mutation and with elevated level of serum  $\text{Ca}^+$  was died of malignant lung cancer, the diagnosis of familial multiple endocrine neoplasia was established.



**Figure 3.** Pedigree diagram of the proband's family. Family members are indicated by generations (Roman numbers) and individuals (Arabic numbers). Circles indicated women and square indicated men. Clinical status was denoted: open symbols, unaffected; solid symbols affected; question mark symbols, undetermined; stippled symbols, mutation carriers without clinical signs; slashed, deceased.

According to previous study, multi-glandular hyperplasia of parathyroid glands is the most common pathologic type in MEN 1. In our patient, only one enlarged parathyroid was detected before surgery but during the operation, two abnormal ones were discovered and the pathology findings confirmed that both of them were hyperplasia. That is explainable because the sensitivity for detecting multi-glandular hyperplasia is limited and suboptimal for all imaging modalities, including sestamibi SPECT. Reported sensitivities for sestamibi in detecting multiglandular hyperplasia is only about 50%<sup>6</sup>. This informs us that even with the help of the preoperation facilities, exploration during operation is very important in treating hyperparathyroidism in MEN 1 patients.

In published articles about pituitary tumors and hyperplasia in MEN 1<sup>7</sup>, the prevalence of pituitary hyperplasia was only 4% among all the MEN 1 patients with pituitary problems. In our case, his pituitary was enlarged and no tumor was detected. This might indicate pituitary hyperplasia but we still need to follow up. There is still no consent on the treatment of MEN 1 related pituitary tumor, and most scholars agree that the treatment procedure should refer to sporadic pituitary tumor. In our patient, after taken bromocriptine, his serum PRL, GH and IGF-1 level all decreased significantly and the enlarged pituitary reduced as well. Although in some assays bromocriptine might be useful in only 10% patients with elevated level of GH<sup>8</sup>, for the MEN 1 patients who may face multi-gland surgeries,

maybe we should still first try bromocriptine and monitor both hormone level and imaging change.

We noticed that the patient's gastrin level was not as high as 5-10 times above the highest normal value according to most diagnostic criteria for gastrinoma. This is the same as Berna et al work<sup>9</sup> and the reason might be earlier diagnosis of gastrinoma in MEN 1 patients than in other types of Zollinger-Ellison syndrome patients. MEN 1 patients may come to hospital because of one chief problem and they might have obscure family history, in that case, we have to pay more attention on the patient's symptoms even if no positive findings in laboratory tests or images are provided. Controversies still exist concerning the treatment for MEN 1 associated pancreatic tumors. One retrospective analysis showed that surgical treatment for tumors < 2 cm had no advantage over conservative treatment<sup>10</sup>. Because our patient suffered 3 operations in one year, and omeprazole can still control his symptoms, he didn't accept surgery. We asked him to do abdominal CT scan every six month.

The patient's low serum K<sup>+</sup> level possibly due to the damage of interstitial nephropathy induced by hypercalcemia, which didn't recover during the follow up. This tells us that MEN1 related hypercalcemia should be treated immediately otherwise the damage can't be reversed.

*MEN1* gene product, menin, is believed to function as a tumor suppressor which has a number of potential roles in maintaining normal cell function. Up to now, 5 functional domains are

known: 3 JunD-interacting domains at codons 1-40, 139-242, and 323-428, and 2 nuclear localization signals at codons 479-497 and 588-608<sup>11</sup>. We identified a novel germline mutation (TCA > TGA) in *MEN1* gene which turns the serine on the 308 codon into stop codon. Accordingly, the menin protein was cut to approximately half of its normal length and lost at least 3 of the 5 functional domains. Previous research<sup>12</sup> have revealed a mutation on codon 308 which also leads to a truncated protein, but that mutation was caused by TCA > TAA. The mutation we identified in this MEN 1 kindred is a novel one.

### Conclusions

We presented a case of MEN 1 in the early stage and followed the progression and treatment results. We also described a novel *MEN1* gene mutation in the patient's kindred which may further extend our knowledge of the variety of genetic abnormalities and deep understanding of the disease on genetic level. Bromocriptine can successfully minimize the patient's enlarged pituitary and decrease hormone level, and this might be associated with the S308X mutation. Maybe more functional and pharmacogenetic studies on this novel mutation should be done in the future.

### Conflict of Interest

The Authors declare that there are no conflicts of interest.

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