A novel mutation of the \textit{MEN1} gene in a Chinese kindred with multiple endocrine neoplasia type 1

Lei Xu, Xu Li, Bo Feng, Yafang Ni, Hua Wang and Lin Wang

Department of Endocrinology, the Affiliated East Hospital, Tongji University, Shanghai 200120, China

\textbf{Abstract.} Germline mutations in the \textit{MEN1} gene are well documented as the genetic cause of multiple endocrine neoplasia type 1 (MEN1). In this study, we performed genetic analysis by direct \textit{MEN1} gene mutation analysis on a Chinese MEN1 family. The two patients in this family were diagnosed as MEN1 by the typical clinical findings of parathyroidoma, insulinoma and pituitary adenoma. The coding sequences, including 9 coding exons and exon/intron boundaries of the \textit{MEN1} gene were amplified by polymerase chain reaction (PCR) and subjected to direct sequencing. Sequence analysis showed a same novel insertion mutation in exon 3 (c.433_434ins CTTC) in both patients, resulting in an open reading frames shift and produced a premature termination codon. None of the other family members had this insert mutation. In conclusion, we add a new mutation of \textit{MEN1} gene in Chinese patients with MEN1, and it would be useful for the diagnosis of the disease.

\textit{Key words}: Multiple endocrine neoplasia type 1, \textit{MEN1} gene, Insertion mutation
Multiple duodenal ulcers were found by gastroscopy. Magnetic resonance image (MRI) of pancreas showed multiple tumors located in the head and tail of the pancreas (Fig. 2A). Bilateral parathyroid tumors were found by computed tomography (CT) (Fig. 2B). Pituitary adenoma was also detected by MRI (Fig. 2C). According to those findings, she was diagnosed with MEN1. The pancreatic tumors were completely removed by surgery. Pathology (Fig. 2D) and immunohistochemistry confirmed that they were insulinomas which could release gastrin. Subsequently, resection of parathyroid tumors was conducted and pathology confirmed the diagnosis of adenoma (Fig. 2E). No treatment for pituitary adenoma had yet been done, but regularly follow-up visits were demanded.

Patient III-4 (Fig. 1), a nephew of the proband, visited our hospital for repeated hypoglycemic attacks at age 18. The laboratory findings for the patient upon admission were summarized in Table 1. Elevated levels of serum Ca, PTH, insulin, TSH and decreased levels of blood glucose were found. A tumor 2.0×1.5cm in size was detected at the pancreatic body by MRI (Fig. 3A), which was removed by surgery then. Pathology (Fig. 3B) and immunohistochemistry showed that it was insulinoma. During the hospital stay, an MRI found pituitary adenomas (Fig. 3C) which might produce and release TSH; it has not been treated and regular follow-up visits were carried on. A CT scan detected a parathyroid tumor (Fig. 3D) then, and the diagnosis of primary hyperparathyroidism was determined according to the laboratory findings. Parathyroid tumor resection was performed and the pathology of the parathyroid tumor was adenoma (2×1.2×0.8cm) (Fig. 3E). After adenoma resec-

---

**Table 1** Laboratory findings in the two patients

<table>
<thead>
<tr>
<th></th>
<th>Reference range</th>
<th>Patient II-5</th>
<th>Patient III-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma Glucose (mmol/L)</td>
<td>3.1-6.4</td>
<td>1.0</td>
<td>2.55</td>
</tr>
<tr>
<td>Insulin (μU/mL)</td>
<td>2.6-24.9</td>
<td>26.22</td>
<td>26.88</td>
</tr>
<tr>
<td>Ca (mmol/L)</td>
<td>2.15-2.55</td>
<td>2.99</td>
<td>2.87</td>
</tr>
<tr>
<td>P (mmol/L)</td>
<td>0.87-1.45</td>
<td>0.82</td>
<td>1.25</td>
</tr>
<tr>
<td>PTH (pg/mL)</td>
<td>15-65</td>
<td>206.2</td>
<td>92.6</td>
</tr>
<tr>
<td>PRL (ng/mL)</td>
<td>3.4-24.1</td>
<td>&gt;470.0</td>
<td>17.5</td>
</tr>
<tr>
<td>TSH (μU/mL)</td>
<td>0.27-4.2</td>
<td>1.22</td>
<td>6.480</td>
</tr>
<tr>
<td>Gastrin (pg/mL)</td>
<td>0-90</td>
<td>136.3</td>
<td>Not available</td>
</tr>
<tr>
<td>GH (ng/mL)</td>
<td>0-10</td>
<td>31.7</td>
<td>2.3</td>
</tr>
<tr>
<td>ACTH (pg/mL)</td>
<td>25-65 pg/mL</td>
<td>Not available</td>
<td>60</td>
</tr>
</tbody>
</table>
A novel mutation of the \textit{MEN1} gene

Fig. 2 Abnormal imaging and pathology for the proband A: Pancreas MRI showed multiple tumors located in the head and tail of the pancreas; B: CT scan showed bilateral parathyroid tumors; C: Pituitary MRI showed a soft tissue tumor in a size of 1.5cm×2.1cm; D: Pathology of resected pancreatic tumor showed tumor cells both in the pancreatic gland and nodule besides pancreatic gland. (HE stain, ×40); E: pathology of resected parathyroid tumor showed parathyroid adenoma. Tumor was well circumscribed, and tumor cells were arranged in cords. (HE stain, ×100); Abnormality was denoted by arrows.
Fig. 3 Abnormal imaging and pathology for the Patient III-4 A: Pancreas MRI showed a tumor at the pancreatic body; B: Pathology of resected pancreatic tumor showed tumor cells and Homer Wright-like structures. (HE stain, ×100); C: Pituitary MRI showed pituitary adenoma; D: CT scan of parathyroid showed a parathyroid tumor in the right parathyroid; E: pathology of resected parathyroid tumor showed parathyroid adenoma, and tumor cells were arranged in solid sheets, follicles and micro cysts. (HE stain, ×100). Abnormality was denoted by arrows.
tion, this patient also had moderate hypercalcemia and slightly elevated serum intact PTH.

These two patients were diagnosed with MEN1. They all had the characteristics of insulinomas, parathyroid adenomas and pituitary tumors. The son of proband exhibited no manifestation of MEN1. The parents of the proband had been deceased for decades. No further information was available about the parents. None of the other family members had any manifestation of MEN1. It is noted that parathyroid adenoma but not its hyperplasia caused hyperparathyroidism in this MEN1 family. Enlargement, albeit highly asymmetric, of multiple parathyroid-glands is usually present in MEN1, but parathyroid adenoma was also reported before that it could cause hyperparathyroidism. Our study confirmed this view.

Participants in this study were informed about the possibility of a genetic study, its implication, and its purpose. A written informed consent was obtained from those wishing to participate in the study. This study was approved by the ethics committee of Shanghai East Hospital.

**DNA extractions and mutation screening**

Venous blood samples were obtained from 8 members of the kindred (4 females and 4 males) for biochemical and genetic analyses. As control, peripheral blood samples were drawn from 25 healthy people who had no relation with this family for MEN1 gene analysis.

Genomic DNA was isolated from peripheral blood leukocytes of the subjects by the phenol/chloroform method. The coding sequences, including 9 coding exons and exon/intron boundaries of the MEN1 gene [3] were amplified by polymerase chain reaction (PCR) and subjected to direct sequencing. Primers are listed in Table 2. PCR was performed in a final volume of 20µL containing 1x HotStarTaq buffer, 2.0 mM Mg2+, 0.2 mM dNTP, 0.2µM of each primer, 1 U HotStarTaq polymerase (Qiagen Inc, CA) and 1 µL template DNA. 1x GC buffer I (TAKARA, JPN) was used for exons 2, 8 and 9 instead of 1x HotStarTaq buffer. The PCR conditions were as follows: 95°C for 15 min; 11 cycles of 94°C for 15 s, 62°C-0.5°C per cycle for 40 s, 72°C for 1 min; 24 cycles of 94°C for 15 s, 57°C for 30 s, 72°C for 1 min; 72°C for 2 min, and 72°C for 90 s (except exon 2, 8 and 9). For exon 2, 8 and 9, The cycling program was 95°C for 15 min; 35 cycles of 96°C for 10 s, 68°C for 4 min. PCR products were purified by SAP (Promega, USA) and Exo I (Epicentre, USA) as per the manufacture’s instructions.

The sequencing reaction was conducted with a BigDye 3.1 kit (Applied Biosystems, Forster City, CA). The cycling program was 96°C for 1 min; 28 cycles of 96°C for 10 s; 50°C for 5 s; 60°C for 4 min. The DNA was sequenced on both strands using an automated DNA sequencer ABI3130XL (Applied Biosystems, Forster City, CA). Primers used were listed in Table 3.

### Results

Germ line mutations of the MEN1 gene were screened in 8 members of this family. One nov-
el insertion mutation was identified in the 2 patients in exon 3 (c.433_434ins CTTC, Fig. 4). This insertion mutation caused open reading frameshift and produced a premature termination codon (p.Ser145ThrfsX41). DNA samples from 25 unrelated normal individuals were also submitted to direct sequencing of exon 3. This insert mutation was absent in the other 6 available relatives and in all unrelated healthy subjects.

**Discussion**

The *MEN1* gene, which was identified in 1997 [3] consists of 10 exons that span approximately > 9 kb of genomic DNA and encodes a 610-amino acid protein referred to as menin. The main transcript of the *MEN1* gene is a 2.8 kb mRNA. Menin, which is ubiquitously expressed, is predominantly a nuclear protein in nondividing cells [6], but in dividing cells it is found mainly in the cytoplasm [7]. Menin has at least 3 nuclear localization signals (NLSs) [8]. The truncated *MEN1* proteins that would result from the nonsense and frameshift mutations, if express, would lack at least 1 of these nuclear localization signals. Menin has been shown to interact with proteins such as JunD, Smad3 family and NFKB family that are involved in transcriptional regulation, genome stability, cell division and proliferation [9, 10].

Different mutations of *MEN1* gene have been identified in familial and sporadic MEN1. Nonsense mutations, frameshift deletions or insertions have been reported in the majority of MEN1 kindred so far studied [11]. These changes are likely to result in major alterations and functional loss of the menin protein and are consistent with the proposed role of the *MEN1* gene as a tumor-suppressor gene [12].
A novel mutation of the MEN1 gene

This study identified a novel insertion mutation of MEN1 gene in a Chinese kindred with MEN1. This insert mutation caused open reading frames shift and produced a premature termination codon (p.Ser145ThrfsX41). These amino acid changes were predicted to result in a truncated and thus inactivated form of menin which lacked the function in suppressing transcriptional activation and controlling cell proliferation. That may drive cells towards inappropriate growth and ultimately result in tumor formation. In agreement with this concept was the absence of the insertion mutation in the other 6 members of this family and 25 unrelated normal individuals.

In conclusion, we have described a novel MEN1 gene mutation in exon 3 in a Chinese kindred. 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 clinical traits.

Acknowledgement

This work supported by the scientific and technological foundation of social development department in Pudong new area, Shanghai, China (foundation number: PW2009B-1).

References