Detection of a Novel Nonsense Mutation of the \textit{MEN1} Gene in a Familial Multiple Endocrine Neoplasia Type 1 Patient and its Screening in the Family Members

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\textbf{Abstract.} We identified a novel nonsense mutation (R29X) of the \textit{MEN1} gene in a familial multiple endocrine neoplasia type 1 (\textit{MEN1}) patient. Molecular analysis of the \textit{MEN1} gene was performed in the family members by a restriction digestion method. The same mutation pattern was seen in both the proband’s younger brother and cousin diagnosed as \textit{MEN1}, and was also observed in the son of the cousin who showed signs of normal levels of serum PTH associated with mild hypercalcemia and hypophosphatemia. These findings suggest that mutation analysis of the \textit{MEN1} gene is very useful in identifying the subclinical state of \textit{MEN1} as well as clinical \textit{MEN1}.

\textbf{Key words:} Multiple endocrine neoplasia type 1, \textit{MEN1} gene, Mutation, Subclinical state

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Fig. 1. Pedigree and phenotypes in this MEN1 family. The proband carrying the 195 C/T mutation in this family is indicated with an arrow. The numbers of subjects are shown below the symbols.

Table 1. The clinical data of cases in MEN1 family

<table>
<thead>
<tr>
<th>(age/sex)</th>
<th>case 1 (68/F)</th>
<th>case 2 (44/M)</th>
<th>case 3 (39/F)</th>
<th>case 4 (37/M)</th>
<th>case 5 (40/F)</th>
<th>case 6 (22/M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Ca (8.2-10.2 mg/dl)</td>
<td>9.8</td>
<td>9.6</td>
<td>10.4↑</td>
<td>10.5↑</td>
<td>10.7↑</td>
<td>10.4↑</td>
</tr>
<tr>
<td>Serum P (2.5-5.5 mg/dl)</td>
<td>4.1</td>
<td>3.6</td>
<td>2.3↓</td>
<td>2.3↓</td>
<td>2.0↓</td>
<td>2.3↓</td>
</tr>
<tr>
<td>Intact PTH (15.0-50.0 pg/ml)</td>
<td>NE</td>
<td>23.0</td>
<td>120.0↑</td>
<td>92.0↑</td>
<td>203.0↑</td>
<td>45.0</td>
</tr>
<tr>
<td>PRL (1.5-9.7 ng/ml)</td>
<td>4.1</td>
<td>1.9</td>
<td>NE</td>
<td>5.9</td>
<td>31.0↑</td>
<td>7.9</td>
</tr>
<tr>
<td>Gastrin (42-200 pg/ml)</td>
<td>NE</td>
<td>76.0</td>
<td>190.0</td>
<td>83.0</td>
<td>130.0</td>
<td>70.0</td>
</tr>
<tr>
<td>Insulin (3.0-15.0 µU/ml)</td>
<td>NE</td>
<td>NE</td>
<td>11.0</td>
<td>12.0</td>
<td>7.0 × 10³↑</td>
<td>NE</td>
</tr>
<tr>
<td>Parathyroidectomy (-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(-)</td>
</tr>
<tr>
<td>Histology of parathyroid NE</td>
<td>NE</td>
<td>Hyperplasia</td>
<td>Hyperplasia</td>
<td>Hyperplasia</td>
<td>Hyperplasia</td>
<td>NE</td>
</tr>
<tr>
<td>Anterior pituitary gland NE</td>
<td>NE</td>
<td>Ne</td>
<td>Non-functioning tumor</td>
<td>Non-functioning tumor</td>
<td>prolactinoma</td>
<td>NE</td>
</tr>
<tr>
<td>Gastro-entero-pancreatic endocrine tissues NE</td>
<td>NE</td>
<td>Non-functioning pancreatic tumor</td>
<td>Non-functioning pancreatic tumor</td>
<td>insulinoma</td>
<td>NE</td>
<td></td>
</tr>
</tbody>
</table>

Other features | Hashimoto’s disease

NE, not examined; ↑, increase; ↓ decrease.

dup for Hashimoto’s disease at Kagawa Medical University since March, 1992. She complained of general fatigue and depression in August, 1997. Blood chemistries revealed hypercalcemia,
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hypophosphatemia and high PTH levels (Table 1). On admission, the imaging study showed a non-functioning pituitary tumor, enlargement of multiple parathyroid glands and non-functioning pancreatic tumor. She underwent a total parathyroidectomy with autoplatination in October, 1997.

In order to evaluate MEN1, her family members shown in Fig. 1 were examined. After obtaining informed consent, blood samples were drawn for measurements of serum calcium, phosphate, several hormones, and DNA analysis. Patient 4 complained of depression, and patient 5 complained of hypoglycemic episodes. Patients 1, 2 and 6 had no physical complaints. Concerning additional family members, the father of patient 6 had acromegaly, urinary calculus and diabetes mellitus, and underwent a pituitary adenomectomy. The history of MEN1 in the grandmother, grandfather, aunt and uncles of the proband is unknown.

Mutation analysis of the MEN1 gene by direct sequence and PCR-restriction fragment of length polymorphism (RFLP)

Genomic DNA was extracted from peripheral blood samples from 4 patients as shown in Table 1 with a DNA extraction kit (Wako Pure Chemical Industries, Ltd, Osaka, Japan). Polymerase chain reaction (PCR) was performed in 25 μl reactions containing 100 ng DNA and 0.25 U of Takara LA Taq (Takara Shuzo Co. Osaka, Japan) according to the manufacturer’s protocol. PCR primer sets were used as others reported [9], and exon 2 through 10 were amplified. Thirty cycles of PCR were carried out in a thermal cycler (Sanko Junyaku, Tokyo, Japan) according to a step program of 98 °C for 20 sec and 66 °C for 180 sec followed by a 15-min extension at 72 ºC. The PCR products were electrophoresed on an 1.0% agarose gel, and the DNA fragments were purified with a QIAquick Gel Extraction kit (Qiagen, Hilden, Germany). The purified DNA fragments were sequenced after the ABI protocol for Taq-dye terminator cycle sequencing on an automated ABI sequencer (Applied Biosystems, Forter City, CA) with an auto-sequence machine. For direct sequencing, the same oligonucleotides were used as for the dideoxy fingerprinting [9].

To confirm and detect the MEN1 gene mutation, the restriction fragment length polymorphism (RFLP) was performed. Genomic DNA (100 ng) was amplified with the oligonucleotides 5'-TTA GCG GAC CCT GGG AGG AGG C-3' and 5'-GAG ACC TTC TTC ACC AGC TCA CGG-3' as described above. Subsequently the PCR product was digested by DdeI (Toyobo Co., Osaka, Japan) according to the manufacturer’s recommendations. The digested sample was electrophoresed on an 1.8% agarose gel containing 0.5 μg/ml ethidium bromide and photographed.

Results

Three family members (cases 3–5) were diagnosed as MEN1 in this family. Cases 1 and 2 had no evidence of MEN1, and case 6 showed normal levels of serum PTH associated with mild hypercalcemia and hypophosphatemia. From the pedigree analysis, the father, uncle and cousin of the proband seemed to be the obligate disease gene carriers (Fig. 1). We performed mutation analysis by direct sequencing for the MEN1 gene, and identified a novel heterozygous mutation of the MEN1 gene in case 3, the proband (Fig. 2A). This C to T mutation at 195 changed the encoded amino acid from arginine to a stop codon (R29X) and introduced a restriction site for the enzyme DdeI (Fig. 2A, B and C). Detections of the MEN1 gene mutation by using PCR-RFLP in 5 other cases are shown in Fig. 2B. This mutation was detected in cases 3–5 diagnosed as MEN1 and was not detected in healthy members (cases 1 and 2). Interestingly this mutation was observed in case 6 which had normal levels of serum PTH.

Discussion

Recent studies have indicated that various mutations of the MEN1 gene have been found in many MEN1 patients and their family members [9–14], suggesting that analysis of this mutation may facilitate early diagnosis of the MEN1. In this study we identified a heterozygous mutation of the MEN1 gene in a case without clinical features of MEN1 as well as clinical MEN1 cases in a Japanese MEN1 family. The screening for MEN1 is important to improve the patient’s prognosis, to
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Fig. 2. Direct sequence and PCR-RFLP analysis of the MEN1 gene in this family. [A] Proband was heterozygous for a C-to-T Transition (arrow) at nucleotide 195, resulting in substitution of an arginine (Arg) for a stop codon. [B] Co-segregation of the MEN1 gene mutation in this family. [C] The restriction map of the wild type (WT) and mutant (mt) sequence is shown. The lane number is the same as the subject number in Fig. 1, and the standard size marker (S) in the form of a 100 base pair (bp) ladder (Pharmacia) is indicated.

A. 

185  
Arg  205
CGAGCTGGCCGAGAGGGCC

Normal

185  
Arg/stop  205
CGAGCTGGCGGAGAGGGCC

C/T

Proband

B. 

S  1  2  3  4  5  6

(bp)

600  457 (WT)  280 (mt)  177 (mt)  300

200

100

C. 

Wild type

mutant

280 bp  177 bp  457 bp

exon 2

Dde I

avoid malignant transformation and bleeding peptic ulcer disease [15]. Usually the clinical screening of MEN1 has been performed by means of biochemical, hormonal, and imaging examinations [16]. Previous studies have reported genetic screening studies in the family members of MEN1 that used polymorphic DNA markers of the MEN1 locus at 11q13, and indicated gene carriers [5-8], but this screening method examines the responsible gene of MEN1 indirectly. On the other hand, we used a direct and simple method to screen for the MEN1 gene mutation. Genetic analysis of the MEN1 gene is a useful tool that allows screening for early diagnosis of MEN1 in family members, when the MEN1 gene mutation is detected in the proband. It seems to be possible to prevent progress of the disease by a combination of genetic examination and early treatment. Four family members including the proband were positive for the MEN1 gene mutation in this study. Case 2, the elder brother of the proband was without mutation and case 4, the younger brother of the proband, was with mutation. This confirms the autosomal dominant inheritance of the MEN1 gene mutation in this family, because the theoretical probability of the gene transfer is 50%.

Various mutations including missense, nonsense and frameshift mutations have been detected with high prevalence in the MEN1 gene and about 40% of them have been observed dominantly in exon 2 [9-14]. We identified a novel heterozygous nonsense mutation (R29X) of the MEN1 gene in this family in exon 2. These observations suggest that exon 2 of the MEN1 gene may be the first target region for diagnosis. On the other hand, an R29X mutation results in the loss of its function, and will lead to MEN1. The function of the MEN1 gene is thought to be that of a tumor suppressor gene at present [9], but the function of the MEN1 gene protein including its domain region is not known. Further study is necessary to analyze its function.

MEN1 phenotypes were different in cases 3, 4 and 5 in the present study, although they had the same mutation of the MEN1 gene. Cases 3 and 4 had a non-functional tumor of both the pituitary and pancreas, but case 5 had pituitary prolactinoma and pancreatic insulinoma. Previous reports have also indicated different phenotypes in family members carrying the same mutation [11, 12, 14],
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and there does not appear to be a direct correlation between genotype and phenotype. These findings suggest that factors other than the MEN1 gene may be involved in the regulation of the MEN1 phenotype expression.

In conclusion, the direct examination of the MEN1 gene germline mutation in MEN1 is very useful in diagnosing MEN1 including the subclinical state of MEN1. The genetic analysis of the MEN1 gene may have clinical applications in the near future.

Acknowledgement

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References