MEN1 Gene Analysis in Patients with Primary Hyperparathyroidism: 10-year Experience of a Single Institution for Thyroid and Parathyroid Care in Japan

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Abstract. Multiple endocrine neoplasia type 1 (MEN1) is an autosomal dominant hereditary disease. Primary hyperparathyroidism is known to occur at an early age in MEN1 patients. In MEN1 patients, special care regarding not only surgery for hyperparathyroidism but also other MEN1-related tumors is required. Between 1998 and 2007, 482 patients, including 16 whose hyperparathyroidism was discovered by family screening for MEN1, underwent surgical therapy for primary hyperparathyroidism at our institution. We recommended MEN1 gene analysis for patients having one of the following clinicopathological features: 1) age younger than 30 years old; 2) enlargement of multiple glands; 3) coexistence or presence of past history of MEN1-related tumors; or 4) family history of hyperparathyroidism or MEN1-related tumors. Sixty patients had at least one of the above features and were recommended for genetic analysis. Thirty-nine of these patients consented to undergo MEN1 genetic analysis and 16 (41%) showed MEN1 mutation. Pathological examination confirmed multiglandular parathyroid hyperplasia in 15 cases. Subject to this strategy, MEN1 index patients in Japan could be detected efficiently and selected for appropriate therapies for hyperparathyroidism and MEN1-related tumors.

Key words: MEN1 gene analysis, Primary hyperparathyroidism (Endocrine Journal 56: 649-656, 2009)

MULTIPLE endocrine neoplasia type 1 (MEN1) is an autosomal dominant hereditary disease that is characterized by the combined occurrence of tumors of the parathyroid, pancreatic islets, and anterior pituitary gland occasionally in combination with several rare tumors such as thymic carcinoid and bronchial carcinoid [1, 2]. The estimated prevalence of MEN1 ranges from 0.01 to 2.5 per one thousand in the general population [2, 3]. The MEN1 gene is located on chromosome 11q13, and its gene product, menin, acts as a tumor suppressor [4-7]. Mutations of the MEN1 gene cause the absence or reduced levels of menin, and loss of menin expression has been identified in tumors in MEN1 patients and mouse models [8-10]. MEN1 has a high degree of penetrance, and the incidences of patients with MEN1 showing clinical evidence were 43% at the age of 20 years, 85% at the age of 36 years, and 94% at the age of 50 years [11].

The most common organ for the clinical manifestation of MEN1 is the parathyroid gland, indicating that the most efficient way to identify index patients with MEN1 is to screen patients with primary hyperparathyroidism. Detection of the MEN1 genetic mutation for patients with hyperparathyroidism is important because the surgical procedure of parathyroidectomy for patients positive for MEN1 mutation differs from that for those negative for MEN1 mutation. In addition, for patients with MEN1 mutation, investigation of whether they have other MEN1-related tumors and constant follow-up to detect newly developed tumors are required. However, genetic analysis of MEN1 is too expensive and time-consuming to perform on all
patients with primary hyperparathyroidism and it is desirable to select only those patients with clinicopathological characteristics that are likely to be seen in MEN1 patients as candidates for MEN1 genetic analysis. Primary hyperparathyroidism in MEN1 patients is most likely due to chief cell hyperplasia and occurs at an early age [12-15]. Therefore, it is suggested that, in order to identify MEN1 index patients, MEN1 genetic analysis is important for patients with hyperparathyroidism at an early age and/or showing enlargement of multiple glands.

In 1998, our institution initiated MEN1 gene analysis to identify MEN1 index patients. We recommended the analysis to patients with hyperparathyroidism when they had at least one of the following clinicopathological features: 1) patients aged younger than 30 years old; 2) enlargement of multiple glands; 3) coexisting or past history of MEN1-related tumors such as pituitary tumor, pancreatic tumor, or thymic carcinoma; or 4) presence of family history of hyperparathyroidism or MEN1-related tumors. In fact, these features are nearly identical to the guidelines published by Brandi et al. in 2001 [16]. This study describes the 10-year experience of our institution with MEN1 gene analysis to identify MEN1 index patients among those with primary hyperparathyroidism. We succeeded in identifying 16 patients with MEN1 mutation using this strategy.

### Patients and Methods

#### Patients

Between 1998 and 2007, 482 patients were diagnosed and underwent surgical therapy for primary hyperparathyroidism. Sixteen of these patients whose hyperparathyroidism was discovered by family screening for MEN1 were excluded from the present study, and the remaining 466 patients were enrolled in this study. These patients were diagnosed with hyperparathyroidism by hypercalcemia, high plasma ionized calcium levels, high intact parathyroid hormone (i-PTH) level, and hypercalciuria. The location of the pathological gland was identified by ultrasonography and Tc-99m MIBI parathyroid scintigraphy. Plain and enhanced CT scans were also adopted for patients whose pathological glands were difficult to identify or those demonstrating enlargement of multiple glands.

<table>
<thead>
<tr>
<th>Exon</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>5’-GACCTGGGTGCGCTTTCTGGAC 3’</td>
<td>5’-GAGGTAGGTTGATGATTTGGAG 3’</td>
</tr>
<tr>
<td>3-6</td>
<td>5’-GTTGGACATTAGGTTGTAACACAG 3’</td>
<td>5’-ACAGTTGAACACAAAGTGAGACTGG 3’</td>
</tr>
<tr>
<td>7-8</td>
<td>5’-CCCTCAGGGCCAGATCTGTAGA 3’</td>
<td>5’-CCATCCCCATCCCCGATACATGC 3’</td>
</tr>
<tr>
<td>9-10</td>
<td>5’-CTGCTAAGGGGTGAGTAAAGAGAC 3’</td>
<td>5’-GGTTTGATACAGACTGTACTCGG 3’</td>
</tr>
</tbody>
</table>

**MEN1** gene mutation analysis was recommended for patients with primary hyperparathyroidism when they had at least one of the following clinicopathological features: 1) aged younger than 30 years old; 2) enlargement of multiple glands; 3) coexisting or past history of MEN1-related tumors such as pituitary tumor, pancreatic tumor, or thymic carcinoma; or 4) presence of family history of hyperparathyroidism or MEN1-related tumors. This study was approved by the institutional review board (IRB) of Kuma Hospital (Kuma Hospital Human Studies Committee #97-0725). All participants in this study gave their prior written informed consent.

**Sequence analysis of the MEN1 gene**

Peripheral blood was obtained from each patient. Genomic DNA was extracted using a DNA extraction kit, Gentorukun (Takara, Shiga, Japan) according to the manufacturer’s protocol. PCR amplification was carried out in 50 μl of PCR mixture containing 1 μM of each primer, 2.5 mM magnesium chloride, 400 ng of extracted DNA, 50 μM deoxynucleotide triphosphates (dNTPs) (Takara), 5.0 μl of LA-PCR Buffer II (Takara), and 2.5 U of LA Taq (Takara). The PCR conditions were 94°C for 3 min, 30 cycles of 98°C for 20 sec and 67°C for 3 min, and final extension at 72°C for 15 min. The PCR products were separated on 1.5% agarose gel and extracted with a Qiaquick Gel Extraction Kit (Qiagen, Tokyo, Japan). Purified fragments were sequenced using a BigDye Terminator Cycle Sequencing FS Ready Reaction Kit with an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Tokyo, Japan) and a sequencing primer. Sequences of the primers are listed in Tables 1 and 2. All primers were purchased from Invitrogen (Tokyo, Japan).
Table 2. Primers for sequencing analysis

<table>
<thead>
<tr>
<th>Exon 2</th>
<th>Exon 7-8</th>
</tr>
</thead>
<tbody>
<tr>
<td>5'-AACCTTAGCGGACCTGGGAGG-3'</td>
<td>5'-GGACTCCTGGGTATCTTCTGTG-3'</td>
</tr>
<tr>
<td>5'-CTACCAACGTCCCGGACTCACC-3'</td>
<td>5'-GGACCAAGGTTGTTGAAACTG-3'</td>
</tr>
<tr>
<td>5'-GAGGTGAGGTGAGTATTTGGAG-3'</td>
<td>5'-AACCACACATCCAGAAGTGG-3'</td>
</tr>
<tr>
<td>5'-AGACCCCTTTCAAGCAGCTACG-3'</td>
<td>5'-TGCTGAGACCCTCCAGAACCCTAC-3'</td>
</tr>
<tr>
<td>5'-GGTGAGCTCGGGAAGCAGTTGAG-3'</td>
<td>5'-CCCATTGCTAACTCCGTAATGC-3'</td>
</tr>
</tbody>
</table>

Exon 3-6

| 5'-GTGTGGGCCCATACTACCTGGC-3' | 5'-CTGCTAGGTTGAGTAAAGGAG-3' |
| 5'-TGCCCCAGAAATGGAGTCCCTTG-3' | 5'-CACCAGAGGAGATGGCAGATGC-3' |
| 5'-GTCATCCCCTGAAGGAGCCAC-3' | 5'-CAGGGTGCTGGAGTTCCAGCCAC-3' |
| 5'-GCTCCCAAGCAAGTCAAGTCGG-3' | 5'-GAAGCTCTGGAGTCTCAGTG-3' |
| 5'-CTTTCCGCTGTCATAACTCCTC-3' | 5'-AAGGGGAGCAGTGTAAGGAGGC-3' |
| 5'-CCTTCCGCTGTCATAACTCCTC-3' | 5'-GGTTGATACAGACTGTACCTCGG-3' |
| 5'-CAAAGTTCTCTTTTCATCGCCCA-3' | 5'-CGCTGGAGAAAGACAGAGTG-3' |
| 5'-GCCCCTCATCTCTCACTCTGG-3' |

Primary hyperthyroidism

![Flow chart for identification of patients with MEN1 mutation and their treatment strategies](chart)

n=466

Age (years) < 30
Enlargement of multiple glands
Coexisting or past history of MEN 1-related tumors
Family history of hyperparathyroidism or MEN 1-related tumors
At least one of the above features

Consent to analysis of MEN1 gene after informed consent

Parathyroidectomy of enlarged gland
Parathyroidectomy of enlarged gland(s)
Total parathyroidectomy with autotransplantation

Selection of patients who have MEN1 gene mutation and the treatment strategies in these cases are summarized in Figure 1. Of 466 patients (482 excluding 16 with MEN1 mutation detected by family screening) in our series, 60 (13%) were regarded as...
Patients 4 and 5, and patients 11 and 12 showed MEN1 mutations at the same sites but there was no blood relationship between these patients. Patient ages ranged from 27 to 67 years (average 46 years). Fifteen patients showed multiple enlarged glands but one patient aged 29 years had a single enlarged gland. Four patients had coexisting or past history of Men1-related tumors (pituitary tumor for 3 patients and pancreas tumor for 1 patient). Four patients had family history for hyperparathyroidism or Men1-related tumors. 

Table 4 indicated the relationship between patient age and MEN1 mutation detected.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Number of patients</th>
<th>Number of patients who recommended gene analysis (%)</th>
<th>Number of patients who underwent gene analysis</th>
<th>MEN1 mutation detected (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>~ 29</td>
<td>24</td>
<td>24 (100)</td>
<td>12</td>
<td>4 (33)</td>
</tr>
<tr>
<td>30 ~ 39</td>
<td>22</td>
<td>10 (45)</td>
<td>7</td>
<td>2 (29)</td>
</tr>
<tr>
<td>40 ~ 49</td>
<td>44</td>
<td>7 (16)</td>
<td>4</td>
<td>2 (50)</td>
</tr>
<tr>
<td>50 ~ 59</td>
<td>147</td>
<td>13 (9)</td>
<td>11</td>
<td>6 (55)</td>
</tr>
<tr>
<td>60 ~</td>
<td>229</td>
<td>6 (3)</td>
<td>5</td>
<td>2 (40)</td>
</tr>
<tr>
<td>Total</td>
<td>466</td>
<td>60</td>
<td>39</td>
<td>16 (41)</td>
</tr>
</tbody>
</table>

*Incidence of MEN1 mutation detected for number of patients who underwent gene analysis.

Table 3 summarizes the profiles of 16 patients with MEN1 mutation. MEN1 mutation sites were located in exons 2, 3, 4, 6, 9, and 10. Mutation sites in patients 1, 3, 4, 5, 8, 9, 11, 12, 15, and 16 have previously been reported in Supplementary Table S1 in reference 17 (also available online at http://www.interscience.wiley.com/jpages/1059-7794/suppmat). Patients 4 and 5, and patients 11 and 12 showed MEN1 mutations at the same sites but there was no blood relationship between these patients. Patient ages ranged from 27 to 67 years (average 46 years). Fifteen patients showed multiple enlarged glands but one patient aged 29 years had a single enlarged gland. Four patients had coexisting or past history of MEN1-related tumors (pituitary tumor for 3 patients and pancreas tumor for 1 patient). Four patients had family history for hyperparathyroidism or MEN1-related tumors.

Table 4 indicated the relationship between MEN1 mutation and patient age. MEN1 mutation analysis was recommended to all patients younger than 30 years. Twelve patients consented to undergo MEN1 mutation analysis and MEN1 mutation was found in 4, accounting for 33% of patients who underwent genetic analysis. Patients 30 years or older were recommended to undergo MEN1 genetic analysis when they had
We followed 16 patients with MEN1 mutations after surgery and the average follow-up period was 73 months (17-135 months). Pancreatic tumors newly occurred in 2 patients during follow-up. To date, MEN1-related tumors occurred in none of 21 patients who were indicated for MEN1 mutation analysis but did not consent to undergo genetic study (follow-up periods: 17-134 months, average 57 months) and in none of 23 who underwent MEN1 mutation analysis but did not show MEN1 mutation (follow-up periods: 17-113 months, average 47 months). None of the 406 patients who were not recommended for MEN1 mutation analysis showed recurrence of hyperparathyroidism or MEN1-related tumors during follow-up (14-137 months, average 81 months).

**Discussion**

It is desirable to detect MEN1 index patients at an early phase because MEN1-related tumors, such as enteropancreatic tumors and thymic carcinoid, are often malignant and life-threatening for MEN1 patients [18, 19]. Hyperparathyroidism caused by hyperplasia is the most common manifestation of MEN1. The surgical design for hyperparathyroidism of patients with MEN1 mutation differs from those without MEN1 mutation. Total parathyroidectomy with autotransplantation or three and a half parathyroidectomy are recom-
mended for hyperparathyroidism of MEN1 patients, while parathyroidectomy of the enlarged glands is performed for patients with parathyroid adenoma without MEN1 mutation. Therefore, it is important to detect index patients with MEN1 efficiently from those who have primary hyperparathyroidism.

In 2001, Brandi et al. published the guidelines for diagnosis and therapy of MEN1 patients and proposed that cases having two or more MEN1-related tumors, multiple parathyroid tumors before age 30, true recurrent hyperparathyroidism, and familial isolated hyperparathyroidism are candidates of MEN1 index patients and indicators for MEN1 germline mutation test [16]. Studies for diagnosis of MEN1 in Japan have also been performed since the 1990s. Hai et al. examined 16 Japanese MEN1 families and identified 40 mutant MEN1 gene carriers [20]. They also found 8 patients having germline mutations of the MEN1 gene by analyzing 20 Japanese sporadic cases with MEN1 [21]. Uchino et al. examined parathyroid tumor specimens from 112 patients and peripheral blood leukocytes from 64 of the 112 patients and detected somatic and germline MEN1 mutations in 25 and 3 patients, respectively [22].

This is the first systematic study for the detection of MEN1 index patients from a large series of hyperparathyroidism patients in Japan. Our institution started MEN1 genetic analysis to detect MEN1 index patients in 1998 for patients with hyperparathyroidism having one or more of the four clinicopathological features indicated above, which are nearly identical to those proposed by Brandi et al. [16]. We ultimately identified 16 MEN1 index patients, accounting for 41% of hyperparathyroidism patients who were recommended and consented to undergo MEN1 genetic analysis. Since the prevalence of MEN1 patients is 2-4% in all cases of primary hyperparathyroidism [22, 23], the detection rate can be considered high. We can conclude that this strategy is very useful to detect MEN1 index patients also in Japan.

It is notable that the age of onset of hyperparathyroidism in MEN1 patients is 20-25 years, that is, 30 years earlier than that due to sporadic parathyroid adenoma [14, 23-25]. Brandi et al. regarded patients having multiple enlarged glands before age 30 as an indicator of MEN1 genetic analysis [16]. We recommended the analysis for all patients younger than 30 years and detected MEN1 mutations in 4 (33%) of 12 patients who underwent the analysis, including one showing single gland enlargement. It is thus suggested that routine MEN1 mutation analysis should be performed for Japanese patients younger than 30 years, regardless of the number of enlarged glands.

Of 15 patients with coexisting or past history of MEN1-related tumors, 8 underwent MEN1 genetic analysis and 4 were diagnosed as having MEN1 mutation. This finding indicates that half of the patients (4 of 8 patients) who were clinically suspected as having MEN1 mutations showed only MEN1 phenocopy. MEN1 phenocopy has already been investigated by some institutions in Western countries and Japan [20, 21, 26-31]. Hai et al. analyzed 12 Japanese patients having MEN1 phenocopy and reported that 4 patients showed multiple enlarged glands and that no lesion other than primary hyperparathyroidism and GH-secreting pituitary tumor developed before 50 years of age [20, 21, 26]. Sakurai et al. showed that the mean age of MEN1 phenocopy patients at diagnosis was 48 years of age, which did not significantly differ from that of probands of familial MEN1 [28]. In our series, all four patients with MEN1 phenocopy had single enlarged parathyroid glands (parathyroid adenoma) and two having histories of pancreatic tumor were younger than 50 years. Therefore, it is impossible to discriminate MEN1 phenocopy from MEN1 based on clinical phenotype, and genetic analysis is required for diagnosis.

There is a limitation of genetic analysis to detect MEN1 patients. MEN1 mutation sites are scattered throughout the whole gene and to date, 1336 MEN1 gene mutations (1133 germline mutations and 203 somatic mutations) have been identified [17]. Missense mutations and in-frame insertions and deletions are often difficult to interpret because of the possibility of polymorphism. In contrast, previous studies demonstrated that MEN1 mutation analysis failed to find MEN1 germline mutation in 10-20% of index cases [22, 32-34]. There is no doubt about the usefulness of MEN1 mutation analysis to confirm a diagnosis of MEN1, but we should also note the limitation of genetic analysis indicated above.

In summary, we showed that 41% of Japanese patients with hyperparathyroidism having one or more of the four characteristics, that is, younger than 30 years, enlargement of multiple glands, coexisting or past history of MEN1-related tumors or a family history of hyperparathyroidism or MEN1-related tumors, showed MEN1 mutation on genetic analysis. Subject
to this strategy, MEN1 index patients could be detected efficiently and selected for appropriate therapies for hyperparathyroidism and MEN1-related tumors.

Acknowledgment

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References


