Screening of the MEN1 Gene and Discovery of Germ-Line and Somatic Mutations in Apparently Sporadic Parathyroid Tumors


Noguchi Thyroid Clinic and Hospital Foundation, Oita 874-0932, Japan

ABSTRACT

Hyperparathyroidism is the first manifestation in a majority of multiple endocrine neoplasia (MEN1) patients. To discriminate between sporadic and hereditary parathyroid tumors and characterize MEN1 somatic mutations, we examined MEN1 gene mutations in patients who had undergone surgery for sporadic parathyroid tumors. DNA was extracted from fresh frozen parathyroid tumor specimens from 112 patients as well as from peripheral blood leukocytes from 64 of the 112 patients. Sequence analysis was performed to examine exons 2–10 of the MEN1 gene for mutations. Loss of heterozygosity (LOH) was also examined by an analysis of codon 418 and 541, which lie within a polymorphic region of MEN1. Somatic MEN1 mutations were found in 25 of the 112 patients (22%). A total of 27 mutations were characterized, 20 of which have not been reported previously. There were 7 nonsense mutations, 10 frameshift mutations, 2 splice site deletions, 5 missense mutations, and 3 in-frame mutations. Nineteen mutations (70%) predicted truncation of the menin protein. Germ-line MEN1 mutations were found in 3 of 64 patients (5%) who had no family history of endocrine tumors associated with MEN1, and these patients were identified as MEN1 gene probands. LOH at the MEN1 locus was detected in three parathyroid tumors showing germ-line mutation. LOH was significantly frequent in parathyroid tumors with somatic MEN1 mutations (15 of 22 tumors, 68%) but not in those without germ-line or somatic MEN1 mutations (14 of 51 tumors, 28%; P = 0.0011). Our findings suggest that alterations of both alleles of the MEN1 gene may be associated not only with endocrine tumors of affected MEN1 patients but also with sporadic parathyroid tumors. Germ-line MEN1 gene analysis can distinguish heritable from nonheritable parathyroid tumors, and MEN1 gene evaluation of patients with apparently sporadic parathyroid tumor is recommended before parathyroid surgery.

INTRODUCTION

Parathyroid adenoma and hyperplasia are commonly found and are the most frequent causes of primary hyperparathyroidism. Hypercalcemia can cause nephro-uro lithiasis, osteoporosis, pancreatitis, and psychiatric disorders. The sporadic form is very common; the hereditary form is also well known. MEN1 is an inherited cancer syndrome characterized by three endocrine tumors in different combinations: (a) parathyroid hyperplasia; (b) pancreatico-gastrointestinal neuroendocrine tumor; and (c) pituitary tumor. Segregation is autosomal dominant, and the overall incidence of hyperparathyroidism is more than 90% in MEN1 patients (1, 2). The prevalence of pancreatico-gastrointestinal tumors and pituitary tumors is 40–70% and 30–60%, respectively. In 1997, germ-line mutations of the MEN1 gene were identified and have been found in members of families afflicted with MEN1 (3, 4). The MEN1 gene consists of 10 exons, and it encodes a putative 610-amino acid (M, 67,000) nuclear/cytoplasmic polypeptide, menin, with two nuclear localization signals (5–8). MEN1 germ-line mutations have been found throughout the coding exons of the MEN1 gene, and no mutational hot spots have been found (9, 10). No genotype-phenotype correlation has been elucidated in MEN1 patients. In the clinical management of MEN1 families, direct molecular analysis of MEN1 gene mutations is replacing conventional genotyping and biochemical screening to discriminate between gene carriers and non-gene carriers.

In sporadic parathyroid tumors, frequent LOH on chromosome 11q13, the chromosome on which the MEN1 gene lies, has also been found (11). Of a small number of parathyroid tumors investigated recently, 15–21% had somatic mutations in the MEN1 gene (12–14). No germ-line mutation was found in these studies. We investigated MEN1 gene mutations in a larger number of patients with apparently sporadic parathyroid tumors and found both germ-line and somatic mutations.

MATERIALS AND METHODS

A total of 112 patients for whom the preoperative diagnosis was sporadic parathyroid tumor underwent parathyroidectomy at Noguchi Thyroid Clinic and Hospital Foundation between 1989 and 1998. The average age of patients was 57.8 ± 13.1 years; 14 patients were men, and 98 were women. Uniglandular disease was diagnosed intraoperatively in 104 patients, and 8 patients were diagnosed with multiglandular disease. The median tumor weight was 758 mg (quartile points, 326 and 1660 mg) in the patients with uniglandular disease and 231.1 mg (quartile points, 52.7 and 624.6 mg) in the patients with multiglandular disease. The histopathological diagnosis, based on parathyroid gland specimens, was parathyroid adenoma in 67 patients, hyperplasia in 44 patients, and adenolipoma in 1 patient. There was no apparent family history of MEN1, hereditary parathyroid tumor, pancreatico-gastrointestinal endocrine tumor, and pituitary tumor at the time of initial evaluation in any patient. A family history of cancer was present in 34 patients, including 2 patients with a family history of pancreatic cancer. No other disease, including tumors of the pancreas or duodenum or pituitary tumor coupled with hyperparathyroidism, was detected in these patients. A history of thyroid disease was present in seven patients, a history of thyroid cancer was present in one patient, a history of nodular goiter was present in one patient, and a history of Graves’ disease was present in five patients. Forty-four patients showed thyroid disease and underwent parathyroidectomy and thyroidectomy. The histopathological diagnosis, based on thyroid gland tissue, was thyroid cancer in 16 patients, adenomatous goiter in 13 patients, follicular adenoma in 12 patients, Graves’ disease in 1 patient, chronic thyroiditis in 1 patient, and malignant lymphoma of the thyroid in 1 patient.

DNA was extracted from fresh frozen parathyroid tumors from 112 patients, and PBLs were extracted from 64 patients as described previously (15). All patients subject to a somatic and/or germ-line analysis gave informed consent before participation in the MEN1 study. Oligonucleotide primers for exons 2–10 of the MEN1 gene were synthesized as described by Lemmens et al. (4). The PCR amplification reaction was carried out in a 50-μl mixture containing 100 ng of template DNA, 1.5 mM MgCl2, 0.2 mM deoxynucleotide triphosphates, 5–10 pmol of each sense and antisense primer, and 1 unit of AmpliTaq Gold (Perkin-Elmer Biosoys, Foster City, CA) with a PROGENE programmable thermal cycler (Techn e, Cambridge, United Kingdom). After initial

Received 12/22/99; accepted 8/2/00.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 To whom requests for reprints should be addressed, at the Noguchi Thyroid Clinic and Hospital Foundation, Noguchi Naka-machi 6-3, Beppu, Oita 874-0932, Japan. Phone: 81-977-21-2151; Fax: 81-977-21-2155; E-mail: uchino@noguchi-med.or.jp.

2 The abbreviations used are: MEN1, multiple endocrine neoplasia type 1; LOH, loss of heterozygosity; PBL, peripheral blood leukocyte.

[CANCER RESEARCH 60, 5553–5557, October 1, 2000]
denaturation at 94°C for 12 min, PCR was carried out for 35 cycles of denaturation for 1 min at 94°C, annealing for 1 min at 55°C to 67°C, and a polymerase reaction for 1 min at 72°C, followed by a 7-min final extension at 72°C.

For nonisotopic cycle sequencing, DNA products were purified using a QIAquick PCR purification kit (Qiagen, Hilden, Germany). These purified products were subjected to an additional 25 PCR cycles with sense or antisense primer by fluorescence-based dideoxy terminator cycle sequencing (Perkin-Elmer Biosystems). These products were then eluted through a centri-sep spin column (Perkin-Elmer Biosystems) and subjected to capillary gel electrophoresis. Data collection and analysis were performed on an automated DNA sequencer (Model 310; Perkin-Elmer Biosystems). All PCR reactions and sequencing were performed repeatedly, and we confirmed the presence or absence of MEN1 mutation.

LOH was investigated at polymorphic sites located at codons 418 (GAC/GAT) and 541 (GCA/ACA). The nonneoplastic counterpart of DNA was available by PBLs from 64 patients. When either exon was heterozygous, the case was judged as informative. In informative cases, when loss of one heterozygous nucleotide was seen in the parathyroid tissue, the tumor was judged as having LOH.

RESULTS

Table 1 summarizes the MEN1 mutation in apparently sporadic parathyroid tumors. Mutations were found in 25 of 112 patients (22%). Two simultaneous mutations were found in two patients: (a) 508del33 and Y341X in patient 21; and (b) 363insT and 1767delT in patient 28. A total of 27 mutations was found; 20 of these mutations had not been reported previously, with the exceptions being R98X, patient 28. A total of 27 mutations was found; 20 of these mutations were in-frame mutations. Nineteen mutations (70%) predicted truncation of the menin protein, and 8 (30%) encoded amino acid substitutions without truncation of the menin protein.
Heterozygosity at codon 541 was found in 19 of 66 (29%) patients. Combining the results of codons 418 and 541, 72 of 112 (64%) patients were informative, and 40 of 112 (36%) patients were not informative in this study. In addition, LOH was judged by the data from mutated locus of two patients with MEN1 mutation in parathyroid tissue. In the present series, LOH was found in 30 of 74 (41%) informative cases, and 16 of 30 (53%) LOH-positive parathyroid tumors had somatic MEN1 mutations. LOH in parathyroid tumors was found in three of three (100%) informative cases with germ-line MEN1 mutations. Significant LOH was found in informative parathyroid tumors with somatic MEN1 mutations (15 of 22 tumors, 68%) as compared with LOH found in informative tumors without MEN1 mutations (14 of 51 tumors, 28%; \( P = 0.0011 \)).

DISCUSSION

This study demonstrates the involvement of MEN1 gene mutations in patients with sporadic parathyroid tumors and reveals germ-line mutation in apparently sporadic parathyroid tumors. Earlier studies have shown MEN1 mutations in some sporadic parathyroid tumors. Heppner et al. (12) reported MEN1 mutations in 7 of 33 (21%) parathyroid tumors, and these tumors also showed LOH. Farnebo et al. (13) reported MEN1 mutations in 6 of 40 (15%) parathyroid tumors. They found that tumors showing LOH and mutation were significantly larger than tumors without LOH and mutation. Carling et al. (14) reported MEN1 mutation in 6 of 13 parathyroid tumors with LOH at 11q13 but reported no significant differences in clinical indices.

In other sporadic endocrine tumors, somatic MEN1 mutation was found in 9 of 27 (33%) gastrinomas and in 2 of 12 (17%) insulinomas (16). In contrast, pituitary tumors had infrequent MEN1 mutations (17–19). No somatic MEN1 mutation was found in adenomas, hyperplasias, and carcinomas of adrenocortical lesions, and germ-line mutation was reported in only one patient with apparently sporadic adrenocortical adenoma (20). In sporadic carcinoid tumors of the lung, 4 of 11 (36%) patients showed somatic MEN1 mutation (21). Although MEN1 gene inactivation may have an important role in the development of endocrine tumors of various organs in patients with MEN1, the difference in the incidence of somatic MEN1 mutation in tumors suggests the involvement of other genes in sporadic endocrine tumor development.

In the present study, no association was found between the somatic MEN1 mutation and clinicopathological parameters in parathyroid tumors. Loss of function of menin protein in sporadic parathyroid tumors is not always associated with multifocality or proliferative activity of parathyroid disease. Although only one patient with multiglandular disease showed MEN1 mutation, three patients initially

Table 2. Clinicopathological parameters of MEN1 mutation-positive and negative parathyroid tumors

<table>
<thead>
<tr>
<th>Parameters</th>
<th>MEN1 mutation Positive (%)</th>
<th>MEN1 mutation Negative (%)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>57.2 ± 13.9</td>
<td>57.9 ± 12.9</td>
<td>0.820(^a)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>2 (14.3)</td>
<td>12 (85.7)</td>
<td>0.732(^b)</td>
</tr>
<tr>
<td>Female</td>
<td>23 (23.5)</td>
<td>75 (76.5)</td>
<td></td>
</tr>
<tr>
<td>Disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uniglandular</td>
<td>24 (23.1)</td>
<td>80 (76.9)</td>
<td>0.681(^b)</td>
</tr>
<tr>
<td>Multiglandular</td>
<td>1 (12.5)</td>
<td>7 (87.5)</td>
<td></td>
</tr>
<tr>
<td>Resected parathyroid gland weight (median)</td>
<td>609.5 (292.4, 1537.4)(^c)</td>
<td>753.1 (304.0, 1636.2)(^c)</td>
<td>0.553(^*)</td>
</tr>
<tr>
<td>Histological diagnosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenoma</td>
<td>15 (22.4)</td>
<td>52 (77.6)</td>
<td>0.926(^a)</td>
</tr>
<tr>
<td>Hyperplasia</td>
<td>10 (22.7)</td>
<td>34 (77.3)</td>
<td></td>
</tr>
<tr>
<td>Adenolipoma</td>
<td>0 (0)</td>
<td>1 (100)</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Statistical significance was calculated by \( t \) test.

\(^b\) Statistical significance was calculated by Fisher’s exact test.

\(^c\) (Lower quartile point, higher quartile point).

\(^*\) Statistical significance was calculated by Pearson’s \( \chi^2 \) test.
diagnosed as having unilobar disease were determined to have germ-line MEN1 mutations and were inadvertently included in the unilobar group. In the absence of any genetic testing, the surgeon determines which parathyroid gland of a patient with hyperparathyroidism is affected either preoperatively or intraoperatively. In MEN1 patients, multiglandular disease occurs. However, there is a wide heterogeneity in size of the parathyroid glands in MEN1 patients and in patients with sporadic primary hyperparathyroidism (22). It is impossible to discriminate perfectly between MEN1 and non-MEN1 patients by clinical features, results of clinical examinations, and macroscopic view of parathyroid glands (23). Multiglandular disease can be missed in centers that do not make a strong effort to identify two and preferably four glands at all initial operations. Genetic testing of the germ-line MEN1 gene or other parathyroid-related genes may predict before surgery whether the parathyroid process is multiglandular or unilobar.

By combining the results of tumor tissue and PBLs in codons 418 and 541, it was possible to obtain 65% informative cases at the MEN1 allele. Codon 541 is a highly polymorphic site among the Japanese, and there is an ethnic difference associated with the allele frequency of this codon (9). LOH was found in 68% of parathyroid tumors showing somatic MEN1 mutation. If two mutations in two individuals (patients 21 and 28) occurred independently in paternal and maternal chromosomes, these individuals also had genetic alterations on both alleles of the MEN1 gene, and 17 of 22 (77%) would have inactivation of both alleles of the MEN1 gene. In the remaining five tumors with somatic mutation but without LOH at the MEN1 locus, nonneoplastic cells may be intermingled with tumor cells. In contrast, in parathyroid tumors without MEN1 mutation, only 28% showed LOH at the MEN1 locus. These results suggest that the MEN1 gene may operate as a tumor suppressor gene, and loss of function of the menin protein may have an important role in the development of parathyroid tumors. In our series, 53% of parathyroid tumors with LOH at the MEN1 allele showed somatic MEN1 mutation. A similar relationship between the frequency of LOH and mutations has been reported previously (12–14). In mutation-negative, LOH-positive tumors, mutations in the noncoding regions of the MEN1 gene, inactivation of the MEN1 gene by methylation, or inactivation of other unknown tumor suppressor gene(s) existing near the MEN1 locus may be present and may play a role in the development of parathyroid tumors (24).

Germ-line MEN1 mutations were found in 3 of 64 (5%) patients with apparently sporadic parathyroid tumors in this study. These three patients showed only one recognized parathyroid gland and underwent a single gland resection. These patients have persistent or recurrent hyperparathyroidism. These patients were genetically diagnosed as MEN1 probands. Examination of the pancreas, pituitary gland, or adrenal gland is necessary. Furthermore, a family study discriminating gene carriers from non-gene carriers is possible. However, after we informed these patients of the results of the genetic diagnosis, they developed serious psychological problems, anxiety about their future, or a distrust of the first operation. This made it difficult to persuade them to undergo reexamination, to undergo subsequent operations for hyperparathyroidism, to undergo exploration to examine the pancreas and pituitary gland, or to have their genetic family screening analyzed. Elucidating germ-line MEN1 mutation before the initial parathyroid surgery is important in these patients. In Japan, the fraction of MEN1 kindreds among all MEN1 probands was small in contrast to the larger fraction seen in Europe and in the United States (1, 10, 25–27). The frequency of undiscovered MEN1 germ-line mutation may differ in each country because this is partly a social issue in certain countries.

A literature review concluded that MEN1 (it was not stated whether this referred to known or occult MEN1) could account for 2–3% among all hyperparathyroidism (28). Muhr et al. (29) reported that clinical examination and hormonal evaluation of 63 patients with hyperparathyroidism did not reveal any signs of endocrine disease suggestive of MEN1. Hyperparathyroidism is the first manifestation of disease in a majority of MEN1 patients, and the age at onset of parathyroid tumor in patients with MEN1 was about 20 years earlier than the age at onset of sporadic parathyroid tumor (1, 2, 30). Bassett et al. (25) calculated age-related penetrances of MEN1. According to their calculations, the age-related penetrance of MEN1 is 52%, 87%, and 98% at 20, 30, and 40 years of age, respectively. To find a new MEN1 family, examination of MEN1 gene mutation before the initial treatment of hyperparathyroidism may be the most effective approach. The total number of nucleotides of the MEN1 gene we must examine, including exon-intron boundaries, is about 2 kb. Moreover, we must divide the MEN1 gene into 10–12 PCR fragments to perform the analysis. At least 1–2 weeks are needed to confirm the presence or absence of MEN1 mutation for each patient. Thus, the present technique for discovering new MEN1 families is time-consuming and expensive. Furthermore, MEN1 mutation cannot be found in 10–15% of MEN1 families. Although these technical and scientific problems are present, preoperative genetic screening of the germ-line MEN1 gene in patients with apparently sporadic parathyroid tumors will be a useful method for discriminating between hereditary and sporadic parathyroid tumors.

REFERENCES


Screening of the MEN1 Gene and Discovery of Germ-Line and Somatic Mutations in Apparently Sporadic Parathyroid Tumors

Shinya Uchino, Shiro Noguchi, Mari Sato, et al.

Cancer Res 2000;60:5553-5557.

Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/60/19/5553

Cited articles
This article cites 27 articles, 7 of which you can access for free at:
http://cancerres.aacrjournals.org/content/60/19/5553.full#ref-list-1

Citing articles
This article has been cited by 1 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/60/19/5553.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.