A Germline Mutation, 1001delC, of the Multiple Endocrine Neoplasia Type 1 (MEN 1) Gene in a Japanese Family

Seiki Wada, Masaki Watanabe, Toshihiko Tsukada*, Shigemitsu Yasuda, Ken Yamaguchi*, Shinji Kitahama, Makoto Iitaka and Shigehiro Katayama

Abstract

Multiple endocrine neoplasia type 1 (MEN 1) is an autosomal dominant inherited disorder characterized by tumors of the enteropancreas, parathyroid and anterior pituitary. The MEN 1 gene was recently cloned, and germline mutations of the gene have been demonstrated in cases of MEN 1. Here, we report a Japanese family with a germline mutation of the MEN 1 gene. The proband (44 y.o., male) had primary hyperparathyroidism (PHP) and pancreatic carcinoid, and his older sister (50 y.o.) had a history of parathyroidectomy for primary hyperparathyroidism at the age of 40. Clinical examination revealed no evidence of PHP or other MEN 1-related tumors in his son. Direct sequencing analysis revealed a heterozygous germline mutation (1001delC) at codon 297 in exon 6 of the MEN 1 gene in the proband and his son. Loss of heterozygosity (LOH) was also found in the resected parathyroid tissue of the proband. The deletion of cytosine 1001 observed in this case induces a frame shift, which causes the appearance of a stop codon (TAG) at codon 367. This mutation appears to be associated with tumors of the endocrine tissues in the cases studied.

Internal Medicine 40: 499-505, 2001

Key words: gastrinoma, hypercalcemia

Introduction

It is well recognized that primary hyperparathyroidism and malignancy constitute the two main causes of hypercalcemia. Primary hyperparathyroidism leads to increased secretion of parathyroid hormone (PTH) from the parathyroid glands; it originates most often from adenoma (~80%), less commonly from hyperplasia (~20%) and very rarely from parathyroid carcinoma (less than 1%) (1). Multiple chromosomal regions have been shown to be missing in parathyroid adenomas, probably reflecting the deletion of tumor suppressor genes (2–4). These chromosomal loci include portions of chromosomes 1p, 6q, 15q and 11q. On chromosome 11, one parathyroid proto-oncogene, the PRAD1 or cyclin D1 gene, was discovered at the breakpoint of an inversion at 11q13 (5). The inversion led to juxtaposition of the regulatory domain of the PTH gene and the DNA encoding cyclin D1. As a consequence, the cyclin D1 gene could be overexpressed (6). Cyclin D1 gene rearrangements have been documented in 5% of parathyroid adenomas. The 11q deletion has been shown to be associated with an autosomal dominant disorder, multiple endocrine neoplasia type 1 (MEN 1) (7). MEN 1 is characterized by tumors of multiple endocrine organs, including the parathyroids, gastrointestinal endocrine tissues and anterior pituitary. The penetration of parathyroid lesions is the highest and is revealed at an early stage of MEN 1 (95% of patients are under 30 y.o.), mostly as biochemical hypercalcemia (8).

The MEN 1 gene has been mapped to chromosome 11q13 and was recently cloned by Chandrasekharappa et al (9). This gene contains 10 exons and encodes a 610-amino-acid polypeptide. Although the physiological role of the protein remains under investigation, it has been speculated to suppress tumorigenesis. Recently various heterozygous germline mutations in the MEN 1 gene have been identified in cases of MEN 1 (10–13). Here, we report a family with MEN 1 who showed a mutation of 1001delC in the proband and his son.

For editorial comment, see p 461.

Case Report

A 44-year-old man was admitted to Saitama Medical School Hospital, referred from another hospital for evaluation and treatment of hypercalcemia and hypergastrinemia. He had a history of duodenal ulcer and type 2 diabetes, which had been treated with an H2 receptor antagonist (famotidine) and a sulfonylurea
Wada et al

glibenclamide). During the follow-up period, he was revealed to have gall bladder (GB) stones and pancreatic tumor by abdominal sonography, with elevation of serum gastrin levels. Although he was operated on for the pancreatic tumor and GB stones, serum gastrin levels remained elevated and hypercalcemia was also noticed. The pathology of the pancreatic tumor was compatible with pancreatic carcinoid tumors (Fig. 1). He was then referred to our department for further evaluation.

Physical examination showed an operation scar at the center of the abdomen. He was 157.8 cm tall with a weight of 79.2 kg. Blood pressure was 122/80 mmHg. The patient was alert and no focal neurological signs were observed. Biochemical examination showed an elevation of transaminases (AST 57 IU/L, ALT 89 IU/L), compatible with the fatty liver observed by abdominal sonography. Serum Ca and alkaline phosphatase levels were elevated to 11.6 mg/dl and 325 (normal range: 96–284) IU/L, respectively. He had an older sister who had undergone surgery for hyperparathyroidism about 10 years previously at the age of 40 (Fig. 2). Since the patient had a pancreatic tumor and hypergastrinemia, biochemical and endocrinological examinations were performed to confirm MEN 1. His serum growth hormone (GH) varied from 0.4 to 26.6 ng/ml with normal values of IGF-1 and IGFBP-3 (Table 1). As shown in Table 2, GH responded unusually to GRH, L-dopa and TRH. Basal values of other anterior pituitary hormones were within the reference range (Table 1). Brain magnetic resonance imaging indicated neither pituitary microadenoma nor significant enlargement of pituitary tissue (Fig. 3). Examination of Ca metabolism showed hypercalcemia and low normal phosphate (2.7 mg/dl; normal range: 2.4–4.4) with an elevated serum intact parathyroid hormone level (100 pg/ml; normal range: 10–65) and a normal value of PTH-related peptide (0.3 pmol/l; normal range: <0.6 pmol/l). Ultrasonography verified enlargement of only a single parathyroid gland. This was also con-

![Figure 1. Endoscopic retrograde cholangiopancreoscopy showing (arrow) impaired pancreatic duct (A), and contrast-enhanced abdominal CT scan (arrow) showing a tumor mass in the tail of the pancreas (B). C: microscopic features of the resected tumor (x200). Tumor cells were positively stained by antibody against pancreatic polypeptides.](image)
Figure 2. Pedigree of family affected by MEN1: males, squares; females, circles. Solid symbols indicate the presence of MEN1-associated tumors: (1) metastatic liver tumor; (3) parathyroid adenomas. Clear symbols represent unaffected family members. The proband (6) is shown by an arrow. The shaded symbol indicates no evidence of MEN1-associated tumors but the presence of the same genetic mutation as that found in the proband.

Table 1. Basal Endocrinological Data

<table>
<thead>
<tr>
<th></th>
<th>Values</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth hormone (GH)</td>
<td>10.3 ng/ml</td>
<td>(&lt;5.0)</td>
</tr>
<tr>
<td>Thyroid-stimulating hormone (TSH)</td>
<td>0.832 μU/ml</td>
<td>(0.43-3.94)</td>
</tr>
<tr>
<td>Luteinizing hormone (LH)</td>
<td>3.4 mIU/ml</td>
<td>(1.8-8.3)</td>
</tr>
<tr>
<td>Follicle-stimulating hormone (FSH)</td>
<td>8.3 mIU/ml</td>
<td>(1.6-11.0)</td>
</tr>
<tr>
<td>Adrenocorticotropin (ACTH)</td>
<td>19 pg/ml</td>
<td>(6.0-36.0)</td>
</tr>
<tr>
<td>Prolactin (PRL)</td>
<td>13.8 ng/ml</td>
<td>(2.7-16.8)</td>
</tr>
<tr>
<td>Somatostatin</td>
<td>28 pg/ml</td>
<td>(1.0-12)</td>
</tr>
<tr>
<td>Insulin-like growth factor (IGF) 1</td>
<td>312 ng/ml</td>
<td>(106-398)</td>
</tr>
<tr>
<td>IGF binding protein 3</td>
<td>2.59 μg/dl</td>
<td>(1.90-3.89)</td>
</tr>
<tr>
<td>Intact PTH</td>
<td>100 pg/ml</td>
<td>(10-65)</td>
</tr>
<tr>
<td>Intact osteocalcin</td>
<td>16 ng/dl</td>
<td>(2.0-13.0)</td>
</tr>
<tr>
<td>Gastrin</td>
<td>385 pg/ml</td>
<td>(42-200)</td>
</tr>
<tr>
<td>C-peptide</td>
<td>1.8 ng/ml</td>
<td>(1.1-3.3)</td>
</tr>
<tr>
<td>Insulin</td>
<td>5.1 μU/ml</td>
<td>(2.0-17)</td>
</tr>
<tr>
<td>Urine C peptide</td>
<td>33.9-43.6 μg/day</td>
<td>(41.0-145.0)</td>
</tr>
</tbody>
</table>

Table 2. Response of GH Secretion to GRH, L-dopa and TRH

<table>
<thead>
<tr>
<th></th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0  30  60  90  120 min</td>
</tr>
<tr>
<td>GRH test</td>
<td></td>
</tr>
<tr>
<td>GH</td>
<td>12.3 19.5 13.3 7.5 3.1</td>
</tr>
<tr>
<td>L-dopa test</td>
<td></td>
</tr>
<tr>
<td>GH</td>
<td>1.4 1.3 13.3 13.0 8.4</td>
</tr>
<tr>
<td>TRH test</td>
<td></td>
</tr>
<tr>
<td>GH</td>
<td>0.5 0.5 0.4 0.4 18.1</td>
</tr>
</tbody>
</table>

GH responses were assessed by administration of GRH (100 μg), L-dopa (500 mg) and TRH (500 μg). GRH: growth hormone-releasing hormone, TRH: thyroid hormone-stimulating hormone-releasing hormone.

firmed by a 99m-technetium-2-methoxyisobutylisonitrile (99mTc-MIBI) scan (Fig. 4) and a 201-thallium-99m technetium-subtraction scan (201TI/99mTc subtraction scan). He was then operated on for parathyroidectomy. Although preoperative localization studies revealed only a single enlarged parathyroid gland, three other mildly enlarged glands were identified and removed. Some fragments of a gland were auto-transplanted to the forearm. He has been treated for the diabetes mellitus and a duodenal ulcer with insulin self-injection (24 units/day) and an H⁺, K⁺-ATPase inhibitor (omeprazole), respectively. He has also been carefully monitored for serum GH, Ca and gastrin levels at outpatient clinics: his gastrin level decreased to 154 pg/ml immediately after parathyroidectomy but the values again increased to 170–242 pg/ml. Informed consent was obtained for genetic analysis of the family, and the proband and his two children (a son and a daughter) agreed to be examined.

DNA sequencing

After obtaining informed consent, DNA was prepared from blood samples with a QIAamp DNA Extraction Kit (Qiagen, Hilden, Germany), and was amplified by the polymerase chain reaction (PCR). PCR amplification of the DNA (~150 ng) was performed in a total volume of 50 μl containing previously reported primer pairs, Taq polymerase (Takara Shuzo Co.,
Figure 3. Magnetic resonance images of the brain in the proband. Frontal T1-weighted (left) and midsagittal Gd-enhanced (right) images reveal no pituitary tumor.

Figure 4. Image of a 99m-technetium-2-methoxyisobutylisonitrile ('mTc-MIBI) scan. The arrow shows a single enlarged parathyroid gland in the proband.

Osaka) and PCR buffer (Takara Shuzo Co.) (10). The initial denaturation was performed for 1 min at 95°C and was followed by 30 cycles of denaturation at 95°C (1 min), annealing at 64–68°C (2 min) and extension at 72°C (1 min) (10). The amplified products were purified with a QIAquick PCR purification kit (Qiagen Inc., Valencia, CA). Nucleotide sequences were determined by direct sequencing of the PCR product using a DNA sequencing kit and an automated DNA sequencer (Dye Terminator Cycle Sequencing Ready Reaction and ABI PRISM 310; Perkin Elmer, Foster City, CA), for both coding and non-coding strands.

**LOH study**

An enlarged parathyroid gland was obtained from the proband at the time of surgery. Fresh tissue was snap frozen in liquid nitrogen and stored at −70°C for molecular analysis. DNA was extracted with a QIAamp tissue kit (Qiagen), amplified by PCR and sequenced directly as described above for genomic DNA.

Microsatellite length polymorphism around the MEN1 locus was analyzed using polymorphic DNA markers on chromosomes 11q13, PYGM, D11S4940 and D11S4946 as described previously (14, 15). PYGM is located centromeric, and D11S4940 and D11S4940 are located telomeric to the MEN1 gene, as reported previously (15). Genomic and tissue DNA were amplified by PCR with a pair of primers, one of which was labelled with fluorescein. The amounts and sizes of the PCR products were determined by electrophoresis, and fluorescence intensity was measured with an automated genetic analyzer (ABI PRISM 310).
1001delC Mutation in MEN 1

Results

Germline mutation analysis
PCR amplification and direct sequencing of genomic DNA extracted from the blood of the proband and his son showed a heterozygous germline deletion of cytosine at position bp 1001 in exon 6 of the MEN 1 gene (Fig. 5). The deletion of 1001C causes a frame shift, which would generate a premature termination codon (TAG) at position 367.

LOH study
LOH was examined in the enlarged hyperplastic parathyroid gland from the proband. Analysis of the tissue DNA revealed that the 1001delC mutant allele was predominant (Fig. 6).
By the age of 40 years, most individuals carrying the gene for MEN 1 become hypercalcemic. It has been shown that chronic hypercalcemia of either parathyroid or non-parathyroid origin can elevate serum gastrin concentrations (17). Changes in serum Ca levels have also been demonstrated to alter serum gastrin levels and gastric acid secretion in MEN 1 (18). Parathyroidectomy of MEN 1 patients resulted in a reduction of serum gastrin levels and gastric acid secretion even when gastrin-producing tumors were present (19). Therefore, it has been suggested that the first treatment option should be parathyroidectomy in patients with Zollinger-Ellison syndrome and hyperparathyroidism. In the present case, successful parathyroidectomy lowered serum Ca levels (from 11.6 to 9.3 mg/dl), which also led to reduced serum gastrin levels (from 310 to 120 pg/ml). Recently it is commonly accepted that autogenous grafting of parathyroid tissue to the forearm is beneficial in the management of parathyroid hyperplasia (20). The high rate of recurrence in MEN 1 patients does not argue against the use of such autogenous grafts (20), since there is a significant advantage in being able to remove the enlarged tumor under local anesthesia. This autogenous graft has been shown to control serum Ca levels more efficiently in patients with MEN 1 (21).

Elevated serum levels of GH are not uncommon in patients with MEN 1. It has been reported that GH-producing pituitary tumors account for approximately one-fourth of pituitary adenomas in MEN 1; these usually develop as multicentric tumors (19). There are also some reports showing that pancreatic or other tumors occasionally produce GRH, which elevates serum GH levels (22, 23). In these cases, plasma GH was either not suppressed or paradoxically increased by an oral glucose load or by TRH administration. In the present patient, basal levels of serum GH varied widely. Even using high resolution MRI, no pituitary lesions were identified (Fig. 3). An authentic GH stimulus of GRH did not significantly modulate serum GH levels. It might be possible that tumors producing GRH remained in the pancreas or duodenal wall, although circulating GRH levels could not be measured in this case. The changes of plasma GH levels are carefully monitored at outpatient clinics.

In conclusion, we identified a germline mutation of the MEN 1 gene in a Japanese MEN 1 family; the proband and his son had a 1001delC mutation. Genetic analysis is helpful for identifying individuals affected by this hereditary disease and for determining the best therapeutic approach.

References