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COMMENT

An Unusual Kindred of the Multiple Endocrine Neoplasia Type 1 (*MEN1*) in Japanese*

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Multiple endocrine neoplasia type 1 (MEN 1) is an autosomal dominant predisposition to hyperplasia/tumor of the parathyroid glands, endocrine pancreas, and anterior pituitary (1). Recently, the *MEN1* gene, which had been mapped to chromosomal region 11q13 (2), was identified by positional cloning (3). The *MEN1* gene, which is composed of 10 exons, encodes a 610-amino acid protein (menin) (3). Germ line mutations of the *MEN1* gene were detected at high frequency in both familial and sporadic cases of MEN 1 in Caucasians (3–8) and in other ethnic groups, including Japanese (9–12).

The prevalence of pituitary adenomas in patients with MEN 1 is very variable, ranging from 15–60% (13–15). This wide range of values presumably depends on different methodological approaches. Of the various subtypes of pituitary adenomas, prolactinomas are most commonly seen in association with the MEN 1, followed by GH-secreting adenomas, ACTH-secreting adenomas, and nonfunctioning adenomas (14–16). In several pedigrees, many affected members with pituitary adenomas were found to have only prolactinomas (17–19). There is a distinct phenotype of MEN 1 in which a prolactinoma and hyperparathyroidism are dominant manifestations and a pancreatic endocrine tumor is rare. The phenotype is termed "the prolactinoma variant of MEN 1" (19–21). The prolactinoma variant of MEN 1, referred to as MEN 1_{Burin}, was described in one Newfoundland family (19, 20), one family from Pacific Northwest (18), and one other family (21). Of 83 affected family members of MEN 1_{Burin}, 93% had parathyroid tumors, 37% had prolactinoma, and 2.5% had gastrinoma (21). One of the eight affected individuals in the Pacific Northwest family, 100% had parathyroid tumors, 75% had prolactinoma, and 12% had gastrinoma (21). Genetic analysis of families from MEN 1_{Burin} and Pacific Northwest disclosed that the patients have the germ line mutation of *MEN1* gene (4, 20). We describe in this study one Japanese unusual MEN 1 kindred in which the four of five mutation carriers were affected and have developed prolactinoma.

Case Report

The pedigree of this family is shown in Fig. 1. The 22-yr-old first sister (II-1) was referred to our department because of secondary amenorrhea and galactorrhea in 1993. Endocrine studies revealed a serum PRL level of 51 μg/L (normal, <15). Magnetic resonance imaging (MRI) demonstrated a less enhancing macroadenoma in the pituitary region. Because the patient could not tolerate bromocriptine therapy because of nausea and vomiting, transsphenoidal adenomectomy was performed. The tumor was diagnosed as sparsely granulated PRL cell adenoma, showing a chromophobic adenoma with immunopositivity only for PRL. After surgery, her menstrual periods resumed and she bore one child. She does not show any parathyroid or pancreatic tumors, even in 1999.

The 23-yr-old second sister (II-2) was also referred to our department because of secondary amenorrhea and galactorrhea in 1995. Endocrine examinations showed moderate hyperprolactinemia (84 μg/L). MRI demonstrated a less enhancing mass lesion, which was confirmed as being a PRL-secreting pituitary adenoma. The patient began receiving bromocriptine therapy (1.25 mg once a day, orally), and serum PRL level decreased to about 2.4 μg/L 2 months later. Because her germ line mutation of the *MEN1* gene was detected, she was reinvestigated in 1999. Her serum levels of intact PTH and calcium were slightly increased (Table 1). Sonographic imaging of the neck detected a likely parathyroid tumor of 12 mm in diameter, and computed tomography (CT) scan of the abdomen demonstrated a pancreatic tumor of 20 mm in the greatest diameter, recently confirmed as a nonfunctioning endocrine tumor (Table 1).

The 46-yr-old aunt (I-3) had secondary amenorrhea at the age of 30 in 1977. At that time, skull roentgenograms revealed enlargement of the sella turcica and destruction of the dor-

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sum sella. Endocrine studies revealed a serum PRL level of 1200 μg/L. The lesion was diagnosed as a prolactinoma. The patient was continuously treated with bromocriptine therapy (2.5 mg twice a day, orally) for 12 yr and bore two children. At the age of 45, a chest CT scan confirmed anterior mediastinal tumor. A thoracotomy revealed histologically a thymic carcinoid tumor. At the age of 46, the patient was admitted to our department because of bitemporal hemianopsia. Endocrine examinations disclosed a serum PRL level of 59 μg/L. MRI demonstrated a well-defined solid mass with suprasellar extension in the pituitary gland. A transsphenoidal pituitary exploration was performed in 1993. The surgical specimen represented a metastatic carcinoid tumor to a PRL-secreting pituitary adenoma. This patient died 4 months after transsphenoidal surgery. She did not show any parathyroid or pancreatic tumors in endocrine tests (Table 1) or CT scan of the neck and abdomen. The clinical course of this case was reported previously (22).

In the third sister (II-3), at the age of 23, the serum PRL level was found to be slightly elevated (56 μg/L). Dynamic MRI demonstrated a microadenoma in the pituitary gland. Two years later, she remained asymptomatic. She was reinvestigated for other lesions in 1999 because of detected germ line mutation of *MEN1* gene. Her serum intact PTH level was slightly increased. Sonographic imaging of the neck detected a likely parathyroid tumor of 8 mm in diameter. CT scan of the abdomen did not disclose any pancreatic tumors. Serum levels of GH, ACTH, TSH, LH, and FSH in the affected members were within the normal range. In the father (I-1) and the mother (I-2), serum levels of anterior pituitary hormones, other endocrine and laboratory studies were within the normal range. Radiological examinations of the mother (I-2), who was 52 yr of age, did not indicate the presence of a pituitary adenoma, parathyroid tumors, or pancreatic tumors.

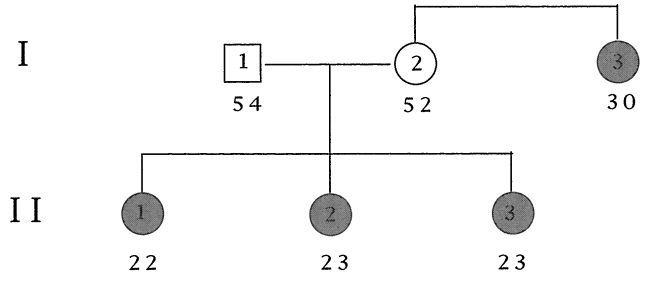

FIG. 1. Pedigree chart of an unusual kindred of MEN 1. Generations available for study are indicated by Roman numerals I and II. Affected subjects are shown in *shaded* symbols. The age at diagnosis is shown below the symbols. ○, Female; □, male; I-1, asymptomatic; I-2, asymptomatic; I-3, prolactinoma and thymic carcinoid; II-1, prolactinoma; II-2, prolactinoma, nonfunctioning pancreatic tumor, and likely parathyroid tumor; II-3, prolactinoma and likely parathyroid tumor.

Materials and Methods

Informed consent for the analyses was obtained from all patients. Biochemical tests and radiological examinations were performed in a consistent way for each patient. The screening program included radiological assessments of pituitary fossa by MRI, parathyroid glands by sonographic imaging, and endocrine pancreas by CT scan, and biochemical assessments of serum PRL, intact PTH, calcium, calcitonin, insulin, glucagon, and gastrin.

Genomic DNA was extracted by standard methods from peripheral leukocytes and the paraffin-embedded thymic carcinoid tumor. The coding sequence, including nine coding exons and 16 splice junctions of the *MEN1* gene (3), of leukocytes DNA from the first sister (II-1) was determined. DNA fragments ranging from 185–260 bp in length were amplified from leukocytes DNA using previously published primers and conditions (12). PCR products were cloned into pCR II vector with a TA cloning kit (Invitrogen, San Diego, CA). DNA sequences of at least six plasmid clones that were amplified in more than two separate experiments were determined in sense and antisense directions, as described previously (23).

To confirm that other family members share the germ line mutations of the *MEN1* gene, the size of PCR products of exon 2B (12) was determined to test for the presence of 4 bp deletion. The forward primer of exon 2B was labeled with 6-FAM fluorescent dye (Perkin-Elmer Corp., Foster City, CA). PCR of 30 cycles was performed with genomic DNAs from leukocytes or the thymic carcinoid as a template. PCR products were subjected to a Model 377 DNA sequencer (Perkin-Elmer Corp.). Data collection and analysis were performed with GENESCAN software (Perkin-Elmer Corp.), as described previously (24).

Results

Germ line mutations of the *MEN1* gene were screened for 12 overlapping PCR products with the corresponding primer sets covering the entire coding region and splice junctions. DNA sequencing of the plasmid clones containing exon 2B from the first sister (II-1) showed heterozygous germ line mutation of the *MEN1* gene, 357del4. This mutation resulted in a frame shift and premature termination of the coding sequence. No mutations in other sets of PCR in the *MEN1* gene were found. PCR products in exon 2B resulted in two

TABLE 1. Endocrine and laboratory studies in this pedigree

	1st sister (II-1)	2nd sister (II-2)	Aunt (I-3)	3rd sister (II-3)	Mother (I-2)	Father (I-1)
PRL (μg/L) (N, <15)	51	84	1200	56	6.6	4.2
Intact PTH (pg/mL) (N, 6.5–59.7)	42	80	47	65	46	40
Calcium (mg/dL) (N, 8.6–11.0)	10.5	11.2	10.6	10.4	10.5	10.3
Calcitonin (pg/mL) (N, <184)	21	21	17	25	28	32
Insulin (pmol/L) (N, 18–72)	22	29	41	25	28	27
Glucagon (pg/mL) (N, 40–180)	112	124	108	115	118	122
Gastrin (pg/mL) (N, <200)	48	54	110	180	75	69

N, Normal range.

The time point in evaluation of serum PRL for each case: II-1, preoperative, at the age of 22; II-2, pretreatment, at the age of 23; I-3, pretreatment, at the age of 30; II-3, asymptomatic, at the age of 23; I-2, asymptomatic, at the age of 52; I-1, asymptomatic, at the age of 54.

The time point in evaluation of serum intact PTH, calcium, calcitonin, insulin, glucagon, and gastrin for each case: II-1, asymptomatic, at the age of 28; II-2, asymptomatic, at the age of 27; I-3, asymptomatic, at the age of 45; II-3, asymptomatic, at the age of 25; I-2, asymptomatic, at the age of 52; I-1, asymptomatic, at the age of 54.

products of 220 bp from the normal allele and 216 bp from the mutant allele. The heterozygous germ line 4-bp deletion was present in the mother (I-2), the first sister (II-1), the second sister (II-2), and the third sister (II-3), but not in the father (I-1) (Fig. 2). The results were confirmed by direct DNA sequencing of PCR products. Although the mother (I-2) has the germ line mutation of the *MEN1* gene, she has been unaffected despite careful examination on MEN 1 phenotype. Because leukocytes DNA from the aunt (I-3) was not available, DNA from the thymic carcinoid was analyzed. Gel electrophoresis showed the PCR products of 216 bp and 220 bp. Reduced intensity (about 25%) of 220 bp compared with that of 216 bp in the tumor was considered indicative of loss of the normal allele. Presence of signal with reduced intensity at 220 bp might be due to the contamination of normal cells in the tumor.

Discussion

MEN 1 is one of the familial cancer syndromes and is characterized by hyperplasia/neoplasia of the parathyroids (90–97%), the endocrine pancreas (30–80%), and the anterior pituitary (15–50%) (16). The patients may have one type or any combination of these three glands involved, but in addition may have other less frequently associated features, such as lipomas, carcinoids, and adrenocortical tumors. MEN 1_{Burin} families have a similar incidence of hyperparathyroidism, but appropriately twice the usual incidence of carcinoid and pituitary tumors compared with families with typical MEN 1, and all of the reported pituitary adenomas have been prolactinomas. The most striking difference between typical MEN 1 and MEN 1_{Burin} is a very low incidence of pancreatic endocrine tumors in the latter syndrome. Because germ line *MEN1* mutations were identified as R460X in a family with MEN 1_{Burin} (20) and Y312X in a family from Pacific Northwest (4), phenotypic differences between typical MEN 1 and MEN 1_{Burin} were not explained only by types of mutations.

Our cases, which were initially considered as isolated familial prolactinomas, later were found to show unusual features of MEN 1 based on the detected germ line mutation and MEN 1 phenotype. Parathyroid tumors are usually the first manifestation of MEN 1 (25, 26). However, prolactinomas were the first lesion diagnosed in this family. The aunt (I-3) suffered from thymic carcinoid along with prolactinoma. Carcinoid tumors are frequently found in MEN 1_{Burin} (19). The second (II-2) and third (II-3) sisters were diagnosed as subclinical primary hyperparathyroidism from the results of serum intact PTH levels and sonographic images. Our cases resembled MEN 1_{Burin} (19, 20) and the MEN 1 family from Pacific Northwest (18), although the second sister (II-2) was found to have a nonfunctioning pancreatic tumor. Thus, although our cases cannot be confirmed as a prolactinoma "variant" of MEN 1 family, these may belong to an unusual prolactinoma "dominant" of MEN 1 family. In our cases, a germ line mutation of the *MEN1* gene was identified as 357del4 in exon 2. Detection of the germ line mutation prompted us to carefully examine MEN 1 phenotypes. Genetic examinations are useful as diagnostic tools for any rare or unusual cases of MEN 1.

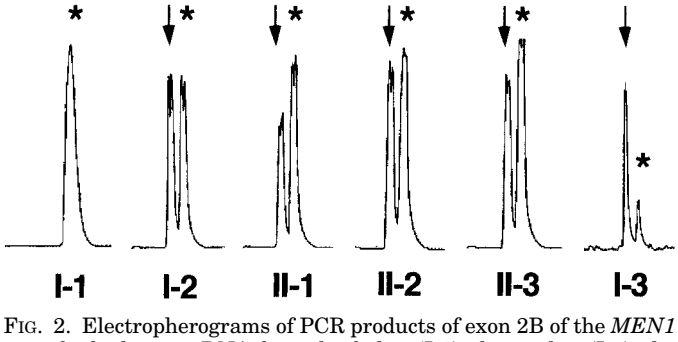

FIG. 2. Electropherograms of PCR products of exon 2B of the *MEN1* gene for leukocytes DNA from the father (I-1), the mother (I-2), the first sister (II-1), the second sister (II-2), the third sister (II-3), and for tumor DNA from the aunt (I-3). *Asterisks* and *arrows* denote 220 bp from the normal allele and 216 bp from the mutant allele, respectively.

The 357del4 yields truncated menin protein with loss of function. Reported deletions of 357del4, 358del4, or 359del4 resulted in the same frame shift. These deletions were encountered frequently in many MEN 1 patients showing typical phenotypes (3, 5, 6, 8, 11). Clinically, there were no prominent features in families with the deletions. In addition, the same somatic deletion in tumors was found in sporadic pancreatic endocrine tumors (27, 28). The DNA region including codons 357, 358, or 359 could be prone to DNA polymerase slippage during DNA replication.

High prevalence of prolactinomas in the prolactinoma variant is not due to the specific mutations. On the other hand, Burgess *et al.* (17) have reported that prolactinomas were not evenly distributed in the Tasman MEN 1 family, being common in some branches of the family while rare in others. Thus, unknown genetic factors other than *MEN1* mutations may contribute to a prolactinoma-dominant phenotype.

Familial pituitary adenomas unrelated to MEN 1 have been reported previously (6, 12, 24, 29, 30). The majority of reported pedigrees were mainly characterized by familial acrogigantism, in which no germ line mutations were found (6, 12, 30). Isolated familial prolactinomas are an extremely rare subset of familial pituitary tumors (7, 29). Germ line mutations of the *MEN1* gene in two families with isolated familial prolactinomas were not found (7), however, clinical information on the two families were scarce in the report.

Recently, it has been reported that inactivation of the *MEN1* tumor suppressor gene, by mutation or by imprinting, does not play a prominent role in sporadic pituitary adenoma pathogenesis (31–33). These results suggest that MEN 1-associated pituitary adenomas develop via genetic pathways that differ from those of most sporadic pituitary adenomas. Molecular basis of penetrance of prolactinomas in the prolactinoma dominant or variant of MEN 1 is unknown. Further analysis of a large set of MEN 1 families, potentially the development of a functional assay for menin and sporadic prolactinoma-specific genetic changes will be necessary to dissolve the dominance of special phenotypes in MEN 1.

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