Endocrine, *vol. 12, no. 3, 223–226, June 2000* 0969–711X/00/12:223–226/$11.00

A Newly Recognized Germline Mutation of *MEN1* Gene Identified in a Patient with Parathyroid Adenoma and Carcinoma

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We report on a patient with primary hyperparathyroidism, owing to the concurrence of parathyroid adenoma with carcinoma, who had a newly recognized germline mutation of the multiple endocrine neoplasia type 1 gene (*MEN1* gene). The patient underwent total parathyroidectomy, and histological examination revealed parathyroid carcinoma and multiple adenoma of the other three glands. Genetic analysis revealed a newly recognized heterozygous germline mutation (842delC, exon 4) of the *MEN1* gene. Both imaging studies and laboratory data showed no evidence of MEN 1 in the patient. Four family members—three sisters and one daughter—had neither clinical features of MEN 1 nor genetic evidence of the *MEN1* gene. This is the first report of a germline mutation of the *MEN1* gene found in a patient who exhibited the concurrence of parathyroid adenoma with carcinoma, suggesting that long-term hyperactivity of the parathyroids may result in the formation of carcinoma.

Key Words: Multiple endocrine neoplasia type 1 gene; mutation; parathyroid; adenoma; carcinoma.

Introduction

Primary hyperparathyroidism (PHP) derives from three different pathological lesions of the parathyroid gland: a single adenoma, multiple adenoma, and carcinoma *(1)*. Most cases of isolated PHP have the adenoma in one or, at the most, two glands. The other associated glands are normal. In familial cases of PHP, such as multiple endocrine neoplasia (MEN), all four parathyroid glands are involved and the histopathological diagnosis is multiple adenoma. Parathyroid carcinoma is extremely rare and represents about 0.5–5% of all cases of PHP *(2–4)*. Nearly all cases of parathyroid carcinoma are isolated, and a single parathyroid gland is involved. Only a few rare cases are reported showing the concurrence of parathyroid hyperplasia with carcinoma *(5,6)*. There are also some familial cases of parathyroid carcinoma including MEN 1 *(7–10)*.

MEN 1 is an autosomal dominant disorder, the classical spectrum of which includes tumors of the parathyroid gland, anterior pituitary, and enteropancreatic region *(11)*. Less frequently observed associations include foregut carcinoids, lipomas, adrenal tumors, and thyroid diseases. PHP is known to occur in more than 95% of MEN 1 patients, and it is usually expressed at an early age. The *MEN1* gene has been mapped to chromosome 11q13 and was recently cloned *(12)*. To date, several germline mutations have been reported in MEN 1 *(13–17)*. There have been no reports of a germline mutation of the *MEN1* gene being identified in a patient with parathyroid carcinoma. We report on a patient with PHP, owing to the concurrence of parathyroid adenoma with carcinoma, who had a newly recognized germline mutation of the *MEN1* gene.

Results

We detected a heterozygous germline mutation of the *MEN1* gene (842delC, exon 4) in the patient (Fig. 1). This point mutation changed the amino acid proline (P) to leucine (L) at amino acid position 245 and caused a frameshift downward to the position in exon 4. We found no germline mutation of the *MEN1* gene in the four other family members (S1, S3, S4, S5 in Fig. 2).

Discussion

The patient underwent total parathyroidectomy, although the preoperative diagnosis was of a thyroid tumor. The pathological diagnosis was multiple adenoma in the three parathyroid glands and parathyroid carcinoma in the one remaining gland. In almost all cases of MEN 1, more than two glands are enlarged in the parathyroid and histological

Received November 5, 1999; Revised October 30,1999; Accepted January 4, 2000.

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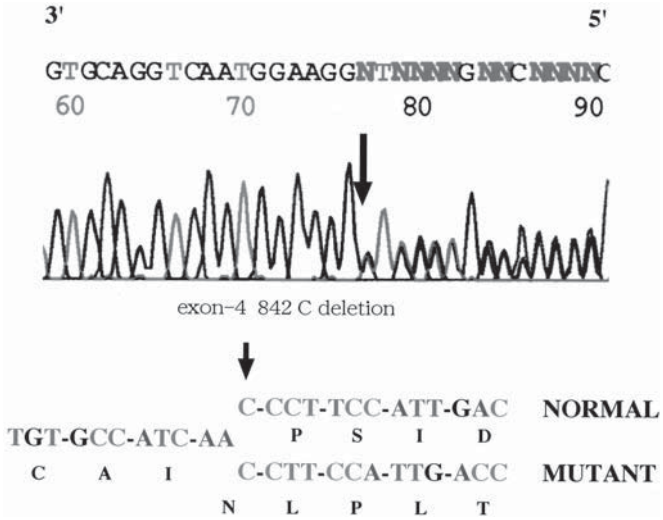


Fig. 1. Direct DNA sequence analysis showing a 1-bp deletion (842delC) at codon 245 in exon 4 of the *MEN1* gene. This mutation changed the amino acid proline (P) to leucine (L) and caused a frameshift from amino acid 245 to 261 in exon 4. The mutation appears in the patient with primary hyperparathyroidism. Arrows indicate the position of the deletions.

findings show multiple adenoma. In isolated PHP, however, a single parathyroid gland is involved and the histological finding is usually of adenoma. For these reasons, we analyzed the *MEN1* gene for germline mutations. After we identified a newly recognized germline mutation of the *MEN1* gene in the patient, we evaluated MEN 1–related lesions. However, the patient had neither pituitary nor pancreatic lesions. Because this patient had a germline mutation of the *MEN1* gene, careful follow-up will be required in the future. Interestingly, our patient presented with the concurrence of parathyroid adenoma with carcinoma. It is not likely that the parathyroid tumor can be ascribed to renal damage, because the disease was not severe enough to produce nitrogen retention at the time of exploration.

There have been several reports indicating the association of parathyroid hyperplasia with malignancy *(5,6)*. Golden et al. *(5)* reported on a 39-yr-old woman who had carcinoma of one parathyroid gland and hyperplasia of two other glands. Two other reports *(8,10)* also described a case of parathyroid carcinoma associated with MEN 1, although a *MEN1* gene mutation was not identified in that case. We raise the question, is parathyroid cancer MEN 1 related or coincidental? Analysis of the loss of heterozygosity on the *MEN1* gene in cancer tissue samples appears to be helpful in answering this question, but such analysis might only provide circumstantial evidence because loss of heterozygosity is reported to exist not only in MEN 1–related tumors but also in sporadic tumors *(18)*. It is therefore suggested that long-term hyperactivity of the parathyroids may result in the formation of carcinoma. We must be careful when dealing with the occurrence of parathyroid cancer in MEN 1 patients.

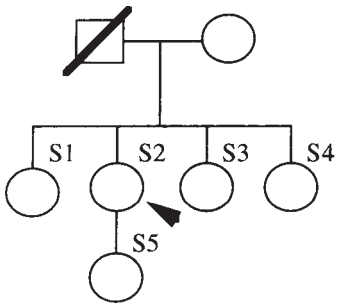
We identified a germline mutation of the *MEN1* gene in the patient, whereas the other four family members, including the patient's three sisters and one daughter had no *MEN1* gene mutation. Laboratory data also showed that they were not affected by MEN 1. Because we had no chance to examine the patient's parents, we could not identify whether this case is sporadic or familial. To date, many germline mutations (more than 80) have been reported in the *MEN1* gene *(13–17)*. The mutations include deletions, nonsense mutations, insertions, and missense mutations, of which deletions are the most common mutations. They are scattered on the entire coding region of the *MEN1* gene from exons 2 to 10, depending on the length of each exon. Exons 2 and 3 are the most common regions for the appearence of mutations, probably owing to their large size, but this does not imply a true "hot spot" as reported in the RET gene of MEN 2 *(19)*. There have been only two types of mutations in exon 4 described in previous reports *(15,16)*, and the mutation (842delC, exon 4) identified in the present case is a newly recognized mutation. This mutation caused a frameshift from amino acid 245 to 261 in exon 4.

There is no direct evidence for the role of *MEN1* gene mutations in the tumorigenesis of MEN 1 because of the lack of information regarding the function and structure of the *MEN1* gene product, menin. Guru et al. *(20)* recently reported that menin is a nuclear protein that has at least two independent nuclear localization signals in the C-terminal portion. They showed that various types of frameshift and nonsense mutations of the *MEN1* gene would result in a truncated menin lacking the nuclear localization signal sequence and would alter the nuclear localization of menin. However, the pathogenesis of missense mutations and frameshift mutations as in our case remains to be clarified. Recently, Agarwal et al. *(21)* reported that menin interacts with the transcription factor JunD. Menin repressed transcriptional activation mediated by JunD. It was also shown that several types of missense mutations disrupted menin interaction with JunD. For example, the missense mutation A242V failed to bind JunD *(21)*. Our case (842delC) causes a frameshift from amino acid 245 to 261, which is very close to the position 242. It is therefore possible that the frameshift mutation identified in our case might interfere with the binding of menin with JunD.

Materials and Methods

Clinical Characteristics of Patient and Her Family Members

In 1985, at the age of 51, the patient initially had an operation for what was considered a thyroid tumor. During the operation, a surgeon noticed that the tumor derived from a right superior gland of the parathyroid and that the other three parathyroid glands were enlarged. The patient therefore underwent total parathyroidectomy with autoplantation. Preoperative values of serum calcium and phosphate were 10.7 and 1.4 mg/dL, respectively.



	Ca (mg/dL)	P (mg/dL)	Intact PTH (pg/mL)	*MEN1* gene mutation
S1	8.9	3.4	26.9	ND
S2	10.7 ↑	1.4 ↓	NE	842delC
S3	8.8	3.2	32.8	ND
S4	8.8	3.5	26.7	ND
S5	9.4	3.5	19.9	ND

Fig. 2. Pedigree of the family. Subjects who had examinations on both PHP and *MEN1* gene mutation are shown as S1, S2, S3, S4, and S5. S2 is the patient with PHP, as indicated by an arrow. Serum levels of calcium (Ca), phosphate (P), intact PTH, and the results of *MEN1* gene mutation tests are shown in the table on the right. Laboratory data were obtained from S2 before parathyroidectomy in 1985. All other laboratory data were obtained in 1999. ND, not detected. NE, not examined. The data of Ca, P, and intact PTH were obtained after S2 underwent a total parathyroidectomy. Up and down arrows indicate the values above and below normal values, respectively.

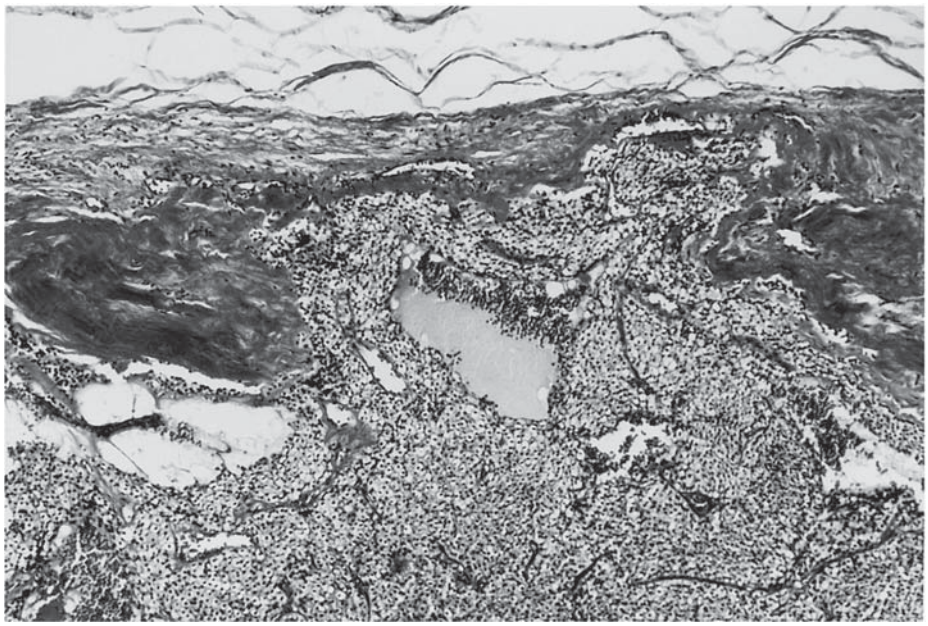


Fig. 3. Light microphotograph of the patient's largest parathyroid tumor showing capsular invasion. Original magnification: × 25.

Pathological examination revealed a parathyroid carcinoma with mitoses in parenchymal cells, nuclear polymorphism, and capsular invasion in the right superior gland (Fig. 3), and multiple adenoma of the other three glands. In 1999, at the age of 59 in 1999, the MEN 1–related lesions were evaluated. Cranial magnetic resonance imaging showed no pituiary lesions and abdominal computed tomography showed no pancreatic lesions. Values of prolactin (PRL) and gastrin were normal (PRL: 7.7 ng/mL; gastrin: 110 pg/mL). Figure 2 presents the pedigree of this family and clinical data regarding PHP. Three sisters and one daughter visited our hospital for family screening in 1999. All of them showed normal values of serum calcium, phosphate, and intact PTH. The patient's father died of unknown causes and the mother was unable to visit our hospital.

Mutation Analysis of MEN1 Gene by Direct DNA Sequencing in Patient and Her Family Members

All participants in this study gave informed consent. Genomic DNA was isolated from the peripheral blood and the *MEN1* gene exons 2–10, including the corresponding splice junction regions, were amplified with a polymerase chain reaction, as previously described *(17)*. The amplified exon products to be sequenced were electrophoresed on a 1.0% agarose gel and purified. DNA sequencing was performed with a Taq FS Dye Termination Cycle Sequencing Kit (Perkin-Elmer), and automated analysis was done with an ABI 377 sequencer, as previously described *(17)*.

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