A Novel MEN1 Gene Mutation in Leukocyte and Parathyroid Tumors of a MEN Type 1 Patient

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A Novel *MEN1* Gene Mutation in Leukocyte and Parathyroid Tumors of a MEN Type 1 Patient

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Abstract:

Multiple endocrine neoplasia type 1 (MEN1) is an inherited genomic disorder involving the *MEN1* tumor suppressor gene. Many germline mutations of the gene have been previously reported. We identified a novel *MEN1* germline mutation in the DNA of a Japanese patient with MEN1. This 48-year-old woman had multiple parathyroid adenomas, a parathyroid carcinoma, a pituitary adenoma, a gastrin-producing islet cell tumor, and multiple gastric carcinoid tumors. Molecular analysis revealed a heterozygous germline mutation: one base T deletion at codon 447 in exon 9 (1451 delT). This case is an isolated incident and none of her relatives was recognized as inheriting MEN1. In the DNA of the resected parathyroid adenoma, only the 1451 delT mutation was detected and an intact allele was missing. The mutation predicts a shift in the reading frame, making a stop signal at codon 457. The mutant gene then will produce defective menin protein with a truncated C-terminal and no nuclear localization signals. These defects seem to make the menin nonfunctional in the nucleus. No intact *MEN1* allele was detected in the parathyroid adenoma and mutation of both *MEN1* alleles might cause tumor growth. It is highly likely that this genetic abnormality causes multiple endocrine tumors in the patient.

Key words: multiple endocrine neoplasia type1, *MEN1* gene, mutation, menin, nuclear localization signal

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Introduction :

Multiple endocrine neoplasia type 1 (MEN1) is an autosomal dominant inherited disorder characterized by tumors of the parathyroid, anterior pituitary, and pancreatic islet cells[1]. Some patients have other associated disorders including thyroid tumors, adrenal cortical tumors, carcinoids of the foregut, lipomas, and ependymomas of the spinal cord[2,3]. Genetic linkage analysis and recombination studies have been used to locate the gene for MEN1 within a small region on chromosome 11q13[4-6] and that gene, named MEN1, was identified by positional cloning[7]. Since then, over 100 heterozygous germline mutations have been confirmed with MEN1families[7-11]. The results from loss of heterozygosity (LOH) analyses and pedigree studies indicate that the gene has a tumor suppressive function[12,13]. This suggests that a second somatic mutation, which occurred in an intact *MEN1* allele of MEN1 patients, would give rise to tumor formation[14].

The *MEN1* gene encodes a 610-amino acid protein called menin, localized primarily in the nucleus. Menin has at least two independent nuclear localization signals in the C-terminal[15]. Loss of these nuclear localization signals by mutations obstructs the nuclear localization of menin, which disrupts its function. Apparently menin represses the transcriptional activation mediated by JunD, a direct menin-interacting transcription factor[16]. Several *MEN1* gene mutations have been found to disrupt menin interaction with JunD, suggesting that the tumor suppressor function of menin involves its binding directly to JunD and inhibiting JunD-activated transcription.

In this study, we report on a middle-aged Japanese woman with MEN1. Direct sequencing studies revealed a novel germline mutation of her *MEN1* gene. The *MEN1* gene in her parathyroid adenoma lost its heterozygosity, suggesting that the mutation was the cause of her MEN1 disorder.

Patient and Methods :

A patient

A 48-year-old Japanese woman was referred to a hospital for treatment of diabetes mellitus. She had no complaints but her serum calcium level of 12.0 mg/dl (normal range 8.4-10.4 mg/dl)and intact parathyroid hormone level of 190 pg/ml(normal range 14-66 pg/ml)were cause for concern. We found hard and fixed masses in the ventral portion of her neck ; computed tomography revealed three parathyroid tumors with surrounding lymphoadenopathy(Fig.1A). An examination of her entire body disclosed a pituitary adenoma, multiple submucosal tumors of the stomach, multiple liver tumors, a pancreatic tumor, and swollen adrenal glands (Figs. 1B-D). Blood tests showed her serum prolactin was elevated to 286ng/ml (normal range 1.4-14.6 ng/ml) while other pituitary hormone levels were within normal ranges. A thyroid-stimulating hormone test showed no response to prolactin. The serum gastrin level was extremely high at 113,000 pg/ml (normal range 37-172 pg/ml) and a secretin test showed a high response to gastrin. These results indicated that she had a prolactin-producing pituitary adenoma and a gastrinoma. Because of their malignant nature, the parathyroid tumors were resected with their surrounding lymph nodes. The subsequent pathological diagnoses were an adenocarcinoma and two adenomas. Cervical lymphoadenopathy proved to be metastasis of the parathyroid carcinoma. Therefore she was diagnosed as having MEN1. Multiple submucosal tumors of the stomach were proven to be gastric carcinoid tumors by an endoscopic transmucosal biopsy. The origin of the multiple liver tumors was not determined.

None of her relatives was recognized as having MEN1. Her parents died from gastric cancer in middle age and one of her half-sisters also died from gastric cancer at a young age. Other relatives showed no remarkable findings of tumorigenesis. The patient is unmarried and has no descendants.

Mutation analysis of the *MEN1* gene by direct sequence

Before examination of the *MEN1* gene, the purpose of the study was explained to the patient and her written informed consent was obtained.

We obtained peripheral blood and a parathyroid adenoma tissue samples from the patient. Genomic DNA was extracted from leukocytes and adenoma tissue with DNA extraction kits (QIAamp Blood Kit and QIAamp Tissue Kit,

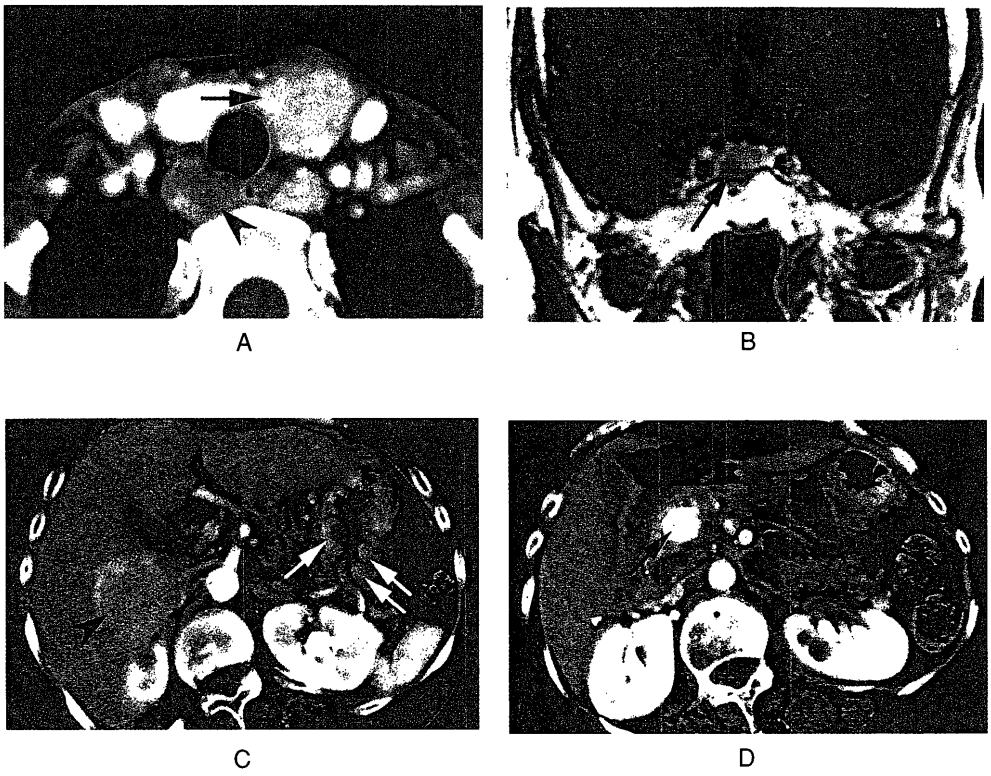

Figure1 Clinical findings
A : An enhanced neck CT showing parathyroid adenoma and a carcinoma. The left superficial tumor was a parathyroid adenocarcinoma (arrow)with lymphoadenopathy of the deep cervical lymph nodes. The parathyroid adenoma was also shown dorsal to the right thyroid glands(arrowhead).
B : An enhanced brain MRI. A pituitary tumor(arrow) was detected.
C : An enhanced abdominal CT. Multiple gastric submucosal tumors(white arrows) and a liver tumor(arrowhead)were shown. Some of the gastric tumors had delle on the top. Multiple disseminated liver tumors were detected in other slices.
D : An enhanced abdominal CT(lower portion).A pancreas head tumor(arrow)was detected.

QIAGEN, Hilden, Germany). Polymerase chain reaction (PCR) amplification was performed on 25-μl aliquots containing 100ng DNA and 0.25U of Taq polymerase (Takara Shuzo Co., Osaka, Japan) according to manufacturer's protocol. Primers were used as previously reported[1] and exons 2 through 10 were amplified. Thirty cycles of amplification were carried out in a thermal cycler (PJ-480, Perkin Elmer, Branchburg, New Jersey USA) with a stepped program of 94℃ for 1 min, 60℃ for 1 min, and 72℃ for 2 min. The amplification products were electrophoresed on a 1.5% agarose gel (SeaKem® GTG® agarose, FMC, Rockland, Maine USA) and the DNA fragments were purified with a QIAquick® Gel Extraction Kit (QIAGEN).

Direct sequencing was performed by the dideoxy method using a commercial sequencing system (Thermosequenase™ pre-mixed cycle sequencing kit, Amersham, Sunnyvale, CA USA) and a DNA sequencer (SQ-5500, Hitachi, Tokyo, Japan). Sequencing primers corresponded to the sense primers of each amplification product, with the 5' end labeled with Texas Red™ tag (Vista systems 5'-oligonucleotide Texas Red labeling kit, Amersham).

Results :

The patient with MEN1 was affected by many tumors in her endocrine organs, which suggested that her *MEN1* gene was involved in a germline genetic disorder. To determine the germline sequences of the *MEN1* gene, we performed direct genetic sequencing on DNA from her leukocytes. From this, we identified a novel heterozygous germline mutation : one base T deletion at the codon 447 (1451 delT) in exon 9 (Fig.2). The one base deletion caused a shift in the reading frame of mRNA transcription, making a stop signal at codon 457. The mutant allele seems to produce a menin protein that is about 25% truncated.

To confirm the relationship between the novel mutation and tumorigenesis, we performed molecular analysis of the *MEN1* gene in the resected parathyroid adenoma tissue. Only the 1451 delT mutant was found, but the intact allele was undetected in the adenoma tissue (Fig.3). Because both an intact allele and the mutant allele of *MEN1* gene were found in the patient germline, an intact allele was apparently deleted with a second somatic mutation, leaving only the mutant allele in the adenoma tissue.

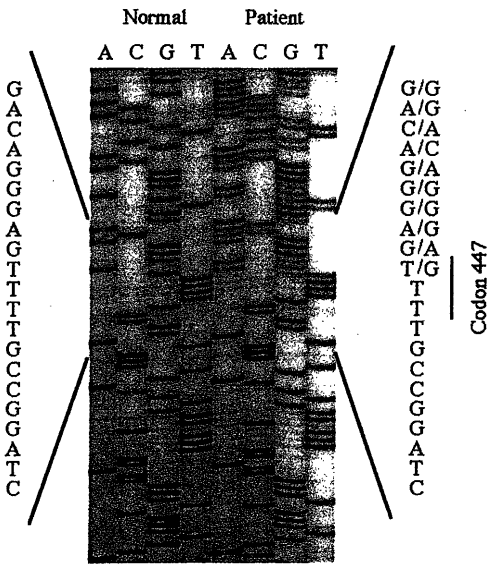

Figure2 Direct sequencing of the germline *MEN1* gene.

The entire coding region of the *MEN1* gene from the patient was examined. Germline sequences show one base deletion at codon 447 in exon 9. The following sequence of the mutant gene, 1451delT, slipped by one base T deletion.

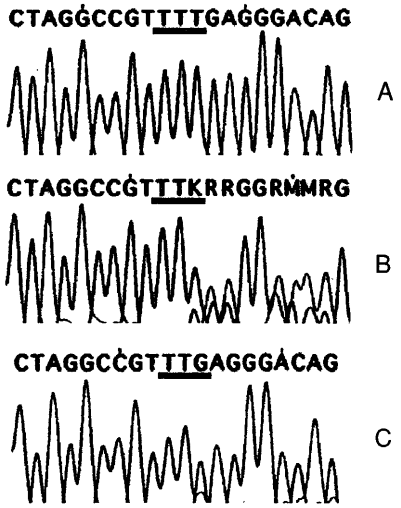

Figure3 *MEN1* gene of parathyroid tumor.

The *MEN1* sequence including codon447 (underlined) were analyzed in leukocytes of a normal subject (A), leukocytes of the patient (B), and the parathyroid adenoma of the patient (C). Genomic DNA of the patient showed heterozygosity, having a single base gap between normal and mutant alleles. In the parathyroid adenoma, the heterozygosity was no longer found, but only the mutant allele appeared. K indicates G and T, R indicates A and G, and M indicates A and C.

Discussion :

We studied a Japanese patient with MEN1 and multiple tumors of her endocrine organs, and we found a novel germline heterozygous mutation of the *MEN1* gene, 1451 delT. This case was suspected to be an isolated incident, because no MEN1 was confirmed among her relatives. It was not clear whether the novel mutation caused the MEN1 but the following facts suggest such a relationship : (i)The 1451 delT mutant gene will produce the menin protein without nuclear localization signals, resulting no tumor suppressive function in the nucleus. (ii)No intact *MEN1* allele was detected in the parathyroid adenoma. As the *MEN1* gene is a tumor suppressor gene, the occurrence of mutation in both alleles is able to cause tumor growth. Disappearance of an intact *MEN1* allele may cause parathyroid cells to grow into a tumor.

Two categories of genes contribute to tumorigenesis : oncogenes and tumor suppressor genes. Oncogenes normally stimulate cell proliferation but their inappropriate activation causes oncogenesis. Tumor suppressor genes normally inhibit cell proliferation and their inactivation causes tumorigenesis. Knudson studied familial retinoblastoma and recognized that patients' tumors could be caused by an inherited germline mutation of a tumor suppressor gene, the retinoblastoma gene *RB1* [17]. The *RB1* mutation exists in all cells. In addition, a somatic mutation of the other intact allele would cause the loss of suppressor functions. These unsuppressed cells grow into tumors in various endocrine organs. Knudson suggests that sporadic retinoblastoma, in contrast to familial retinoblastoma, could arise after somatic inactive mutations of both *RB1* gene in a single cell. These two mutations occur postnatally in a single cell with the low frequency, resulting the unilateral location and the late age onset of the tumor in the sporadic form of the disease.

The *MEN1* gene is also a tumor suppressor gene and its mutations have been detected in many cases of MEN1 and related diseases. Inactivation of both *MEN1* alleles in a single cell would cause the loss of tumor suppressive function as in the *RB1* gene. Our patient with MEN1 was affected in middle age and developed a great number of endocrine tumors. Therefore, we speculated that the patient had a germline disorder of the *MEN1* gene. The molecular analysis of the patient's DNA showed a novel heterozygous *MEN 1* gene mutation in the germline sequence.

The function of the *MEN1* gene is practically unknown. It was known that the encoded menin protein has at least

two independent nuclear localization signals on its C-terminal[15]. Nuclear localization signals are a polybasic motif and are necessary for nuclear targeting[18,19]. Proteins lacking these signals are unable to translocation into the nucleus nor exert their function. Many nonsense mutations and deletions have been detected in the genes of patients with MEN1. Most of these mutations cause a truncation of the menin C-terminal, including the nuclear localization signals[20]. The 1451 delT mutation will cause a stop signal at codon 457, resulting in the formation of cause the mutant gene products without both nuclear localization signals. It is likely that the mutation obstructs the translocation of menin to the nucleus and the resulting tumor suppressive action there.

Supporting data were obtained from the molecular analysis of DNA from the patient's parathyroid adenoma. The *MEN1* gene is a tumor suppressor gene and dysfunction of both alleles causes tumorigenesis in endocrine organs. If the heterogeneous germline mutation, 1451 delT, is the underlying cause of MEN1, then the intact allele of the *MEN1* gene ought to be unworked in the adenoma. Analysis by direct *MEN1* gene sequencing showed only the 1451 delT mutant allele in the parathyroid adenoma. An intact allele was apparently deleted by a somatic mutation, leaving only a mutant allele in the parathyroid cell. The loss of the MEN 1 gene's tumor suppressive function from germline and secondary somatic mutations would give rise to tumor formation in a patient's parathyroid cells.

In conclusion, we studied an isolated case of a Japanese patient with MEN1 and various endocrine tumors. Molecular analysis of the patient's *MEN1* gene disclosed a novel germline mutation, 1451 delT. In DNA from the parathyroid adenoma, an intact allele of the gene was missing and only the mutant allele was detected. Our studies suggest that this genetic abnormality causes tumor formation in patients with MEN1.

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