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Different Phenotypes of Multiple Endocrine Neoplasia Type 1 (MEN1) in Monozygotic Twins Found in a Japanese MEN1 Family with MEN1 Gene Mutation

HIROYOSHI NAMIHIRA, MAKOTO SATO, AKIRA MIYAUCHI*, HIDEMI OHYE, SHUJI MATSUBARA, MOHAMMAD MR BHUIYAN, KOJI MURAO, SETSUKO AMENO**, KIYOSHI AMENO**, IWAO IJIRI** AND JIRO TAKAHARA

First Department of Internal Medicine, Kagawa Medical University, 1750–1 Ikenobe, Miki-cho, Kita-gun, Kagawa 761–0793, Japan

** Kuma Hospital, 8–2–35 Shimoyamate-dori, Chuo-ku, Kobe 650–0011, Japan*

*** Department of Forensic Medicine, Kagawa Medical University, 1750–1 Ikenobe, Miki-cho, Kita-gun, Kagawa 761–0793, Japan*

Abstract. We report monozygotic twins who showed different MEN1 phenotypes. The proband (28 y.o., female) had both primary hyperparathyroidism (PHP) and insulinoma, and genetic analysis revealed a point mutation (569del1, exon 3) of the *MEN1* gene. This mutation causes a frameshift and produces a stop codon at codon 184. Restriction digestion (HinfI) analysis confirmed the same mutation of the MEN1 gene in six of the affected members including her two sisters, the monozygotic twins, and no such mutation in two unaffected members. In two generations of this family, eight of eleven family members had PHP and four of them were found to have other MEN1-related lesions. Both of the monozygotic twins had PHP. Interestingly, one had pancreatic tumor but the other had no evidence of it. Pituitary MRI showed no pituitary lesion in either of them. This is the first Japanese case of monozygotic twins with different MEN1 phenotypes.

Key words: MEN1, Monozygotic, Twin, Japanese, Mutation

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MULTIPLE endocrine neoplasia type 1 (MEN1) is an autosomal dominant disorder, the classical spectrum of which includes tumors of the parathyroid gland, anterior pituitary, and enteropancreatic region. Less frequently observed associations include foregut carcinoids, lipomas, adrenal tumors, and thyroid diseases. Parathyoid tumors are known to occur in more than 95% of MEN1 patients and primary hyperparathyroidism (PHP) is usually expressed at an early age [1, 2]. The incidence of other MEN1-related lesions differs in various reports. Prolactinoma and gastrinoma are commonly found as lesions of the pituitary and endocrine pancreas, respectively, in MEN1.

The *MEN1* gene has been mapped to chromosome 11q13 and recently cloned [3, 4]. This gene encodes a polypeptide (menin) of 610 amino acid residues, which show no homology with other functional-proteins. Loss of heterozygosity on chromosome 11q13 frequently occurs in the neoplasms of affected patients, and heterozygous germline mutations of the MEN1 gene have been identified in MEN1 patients [3, 4]. Recent reports indicate a high incidence of MEN1 gene mutations in American [5], European [6], and Japanese [7] MEN1 patients. In these previous reports, genotype-phenotype correlations are not evident among the families or even within each family. We report here a Japanese MEN1

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Correspondence to: Makoto SATO, M.D., First Department of Internal Medicine, Kagawa Medical University, 1750–1, Ikenobe, Miki-cho, Kita-gun, Kagawa, Japan

family including monozygotic twins who showed different MEN1 phenotypes.

Subjects and Methods

Clinical characteristics of the MEN1 family

The pedigree of this family is shown in Fig. 1 and the clinical data on eight selected family members in Table 1. Case 3 (28 y.o. female), who was the proband of this family, underwent partial pancreatectomy for insulinoma at the age of 27. At the age of 28, she was diagnosed as having primary hyperparathyroidism (PHP) and subsequently underwent parathyroidectomy at Kuma Hospital. Cranial magnetic resonance imaging (MRI) showed no pituitary lesion. Cases 4 and 5, who are monozygotic twins, visited Kuma Hospital for family screening. Case 4 was diagnosed as having PHP. Abdominal computed tomography (CT) showed a pancreatic tumor (1.5 cm in diameter) in the head of pancreas. Although her serum gastrin level was slightly elevated, other pancreatic hormones (insulin, glucagon, secretin) were normal. The pancreatic tumor was therefore diagnosed as non-functioning tumor. Cranial MRI showed no pituitary lesion in case 4. Case 5 also had PHP, but neither pituitary tumor nor pancreatic tumor was evident. The proband's father complained of recurrent urolithiasis, but he refused to have any clinical examinations. The grandfather of the proband died from an epileptic coma. Case 1 had suffered from a gastro-duodenal ulcer since the age of 32, and was diagnosed as having Zollinger-Ellison syndrome at the age of 48 and was treated with an H2-blocker. At the age of 53, she was diagnosed as having PHP and underwent parathyroidectomy at Kuma Hospital. Cranial MRI showed no pituitary lesion. Case 2 was diagnosed as having prolactinoma at the age of 40 and was treated with bromocriptine. She underwent a partial pancreatectomy for insulinoma at the age of 45. She was diagnosed as having PHP at the age of 50 and is awaiting parathyroidectomy at Kuma Hospital. Her brother underwent parathyroidectomy for PHP at the age of 42. Case 8, the daughter of case 2, visited Kuma Hospital for our family screening and was diagnosed as having PHP at the age of 28. Both cases

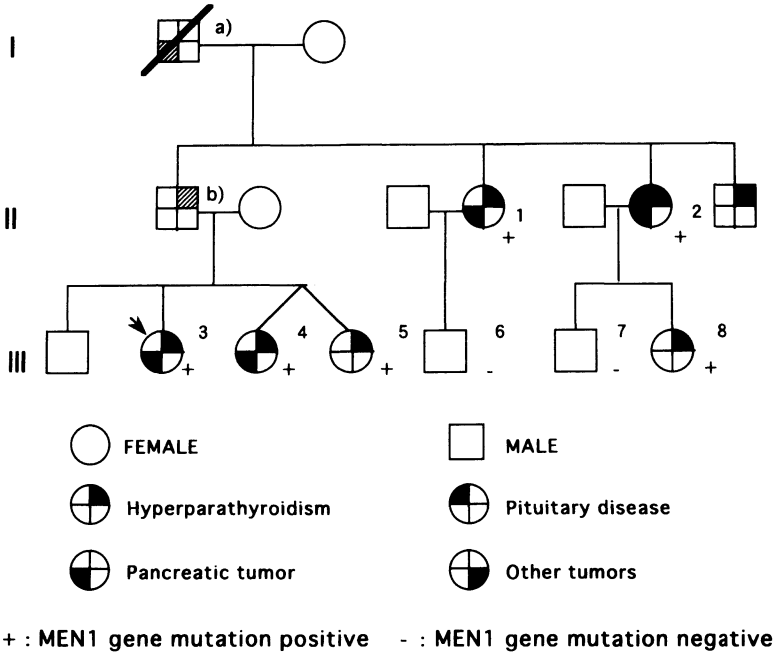

Fig. 1. Pedigree and phenotypes of this MEN1 family. The proband carrying the 569 deletion in this family is indicated as an arrow. The number of subjects is shown below the symbols. Cases 4 and 5 are monozygotic twins. Cases a and b were not examined. Case a died from an epileptic coma and case b complained of recurrent urolithiasis. These cases are suspected of insulinoma and hyperparathyroidism shown as shaded boxes, respectively.

Table 1. Clinical data of cases in MEN 1 family

(age/sex)	case 1 (53/F)	case 2 (50/F)	case 3 (28/F)	case 4 (26/F)	case 5 (26/F)	case 6 (31/M)	case 7 (29/M)	case 8 (28/F)
(normal value)								
Serum Ca (8.5–10.1 mg/dl)	11.0 ↑	10.8 ↑	10.8 ↑	10.1 ↑	10.4 ↑	9.7	9.2	10.3 ↑
Serum P (2.4–4.3 mg/dl)	1.8 ↓	2.9	2.7	2.4 ↓	1.9 ↓	3.5	3.6	3.1
Intact PTH (10–65 pg/ml)	361.0 ↑	169.0 ↑	76.9 ↑	87.8 ↑	125.0 ↑	38.3	26.3	83.3 ↑
PRL (M 1.5–9.7 ng/ml) (F 1.4–14.6 ng/ml)	<1	NE	7.8	5.0	7.7	NE	NE	NE
Gastrin (<200 pg/ml)	17000 ↑	NE	45	240 ↑	ND	NE	NE	NE
Parathyroidectomy	(+)	(−)	(+)	(−)	(−)	(−)	(−)	(−)
Anterior pituitary gland tumor	ND	Prolactinoma	ND	ND	ND	NE	NE	ND
Gastro-entero-pancreatic tumor	Gastrinoma	Insulinoma	Insulinoma	NFT	ND	NE	NE	ND

ND: not detected NFT: non-functioning tumor
NE: not examined

6 and 7 also visited Kuma Hospital for our family screening and neither showed any traces of PHP. All participants in this study signed an informed consent form.

Mutation analysis of the MEN1 gene by direct DNA sequencing and genetic analysis of DNA markers

Genomic DNA was isolated from the blood of case 3 with a DNA Extraction Kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan). The MEN1 exons 2–10, including the corresponding splice junction regions, were amplified with a polymerase chain reaction (PCR), as previously described [7]. The amplified exon products to be sequenced were electrophoresed on a 1.0% agarose gel and purified using a QIA Quick Gel Extraction Kit (QIAGEN Inc., Valencia, CA). 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 [7]. A mutation found in case 3 was analysed in the genomic DNA isolated from cases 1, 2, 4, 5, 6, 7, and 8. The DNA was amplified with the oligonucleotides 5′-CATGTTAAAGCACAGAGGACC-C-3′ and 5′-CCACAGCAAAGGCCACACCGGA-GAT-3 (underline; mismatched nucleotide). The PCR product was digested with HinfI (Toyobo Co., Osaka, Japan) according to the manufacturer's instructions. Samples were electrophoresed in a 4.0% agarose gel containing ethidium bromide and visualized with a UV transilluminator. Seven DNA markers were examined to prove that cases 4 and 5 are monozygotic twins. The markers, C2249G, C1364T, and 2248del3, are benign polymorphisms in MEN1 gene and the genetic patterns were examined as described previously [7]. The markers, LDLR, CYPA, HBGG, D7S8, and GC, are genetic markers located in chromosomes 19p13.1–13.3, 4q28–31, 11p15.5, 7q22–31.1, and 4q11–13, respectively and the genetic patterns were examined with a commercial kit (AmpliType, Perkin-Elmer).

Results

We detected a heterozygous germline mutation in the MEN1 gene (569C del) in exon 3 (Fig. 2). This mutation caused a frameshift and produced a stop codon at amino acid position 184. This mutation neither created nor abolished a restriction site in

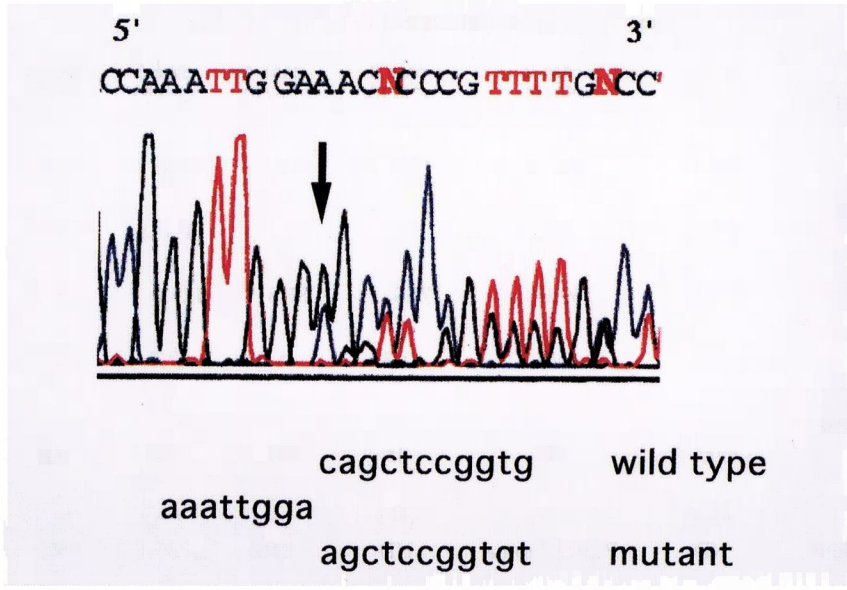

Fig. 2. Direct sequencing in case 3. The result shows a 1 bp deletion at the third position (GAC) of codon 153 (arrow). The deletion causes a frameshift that continues to codon 184 and produces a stop codon. The mutation sequence creates a primer-introduced HinfI site (GACATC to GAATC).

any commercially available enzymes. Thus, PCR primer-introduced restriction analysis was carried out as previously reported [7]. The mutant sequence created a primer-introduced HinfI site (GACATC to GAATC) and the digest had three fragments, 86, 60, and 26 bp fragments in the affected members with heterozygous MEN1 gene mutation (Fig. 3). The mutant 60 bp fragments were found in the proband (case 3) and also in cases 1, 2, 4, 5, and 8 (Fig. 3). In cases 6 and 7, however, only normal 86 bp fragments were found. These results were completely in accordance with the onset of PHP in this family. Seven different genetic markers were identical in cases 4 and 5 (Table 2) suggesting that they are monozygotic twins.

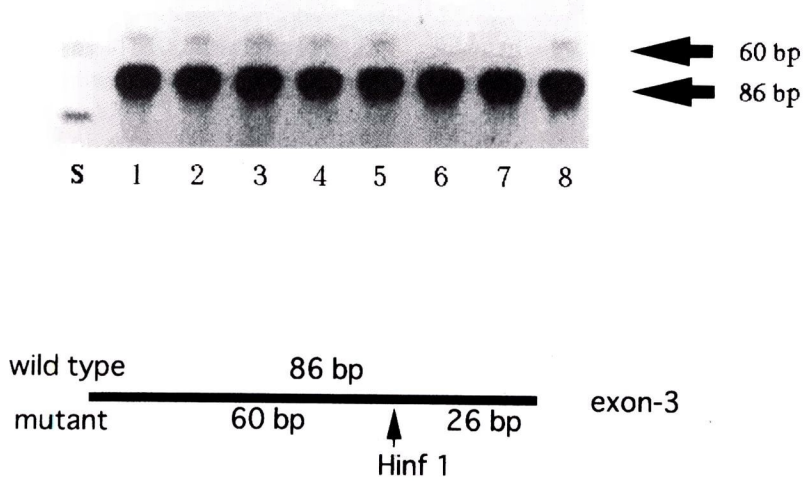

Fig. 3. Restriction endonuclease analysis of eight family members. The lane number is the same as the subject number in Fig. 1, and (s) in the form of ϕX174/HaeIII digest is indicated. Digestion with HinfI shows the presence of 60 and 26 bp and the absence of 86 bp in the mutant allele.

Table 2. Genetic markers in monozygotic twins (case 4 and 5)

	C2249G	C1364T	2248del3	LDLR	CYPA	HBGG	D7S8	GC
case 4	C/C	C/T	del(−)/(−)	B/B	A/A	A/A	A/B	B/C
case 5	C/C	C/T	del(−)/(−)	B/B	A/A	A/A	A/B	B/C

The markers, C2249G, C1364T, and 2248del3 are benign polymorphisms in MEN1 gene (ref 7). The markers, LDLR, CYPA, HBGG, D7S8, and GC, are genetic markers located in the chromosome 19p13.1–13.3, 4q28–31, 11p15.5, 7q22–31.1, and 4q11–13, respectively. All genetic markers are identical in these two cases.

Discussion

This family includes seven MEN1 patients and one suspected case (the proband's father). Three of the seven MEN1 patients were diagnosed on the basis of our family screening, after the proband was diagnosed as having MEN1 and the others were diagnosed separately based on the clinical symptoms due to the MEN1-related lesions. All seven patients had PHP and one family member is highly suspected of having PHP as he has recurrent urolithiasis. One patient had gastrinoma and another two had insulinoma. One patient, who had insulinoma had a prolactinoma. In MEN1, the incidence of PHP is known to be more than 95% [1, 2, 8–11]. Gastrinoma and insulinoma are common enteropancreatic lesions in MEN1, and prolactinoma is also a common pituitary lesion. In this family therefore, the affected members seem to show the typical MEN1 phenotypes.

Genetic analysis identified a point mutation of the MEN1 gene in the proband (case 3) and confirmed the mutation in six affected members who had MEN1-related lesions. This mutation was negative in the two family members who had no PHP. The mutation is therefore completely in accordance with the occurrence of MEN1 in this family. Although the mutation is the same in this family, the MEN1 phenotypes differ among the affected members. In the monozygotic twins, who are sisters of the proband, a pancreatic tumor (non-functioning tumor) was found only in the elder one. This is the first Japanese case in which monozygotic twins show different MEN1 phenotypes. There have been two other reports indicating that monozygotic twins show different MEN1 phenotypes, although MEN1 gene mutations were not identified in these cases [12, 13]. Since the genetic background is identical between these monozygotic twins, a somatic event may be important in characterizing the MEN1 phenotypes. However, the occurrence of MEN1 lesions depends on the age and the age-related penetrances are about 80% in the present case (26 y.o.) [11]. Therefore, we cannot rule out the possibility that they may show the same MEN1 phenotypes in the future.

A tumor suppressor role for the MEN1 gene has been suggested, based on Knudson's hypothesis, named the two-hit theory, that hereditary cancers develop because of the inheritance of a mutated tumor suppressor gene (first hit) and that a somatic mutational event (second hit), involving the wild type allele of the gene, leads to the neoplasia [14]. Indeed, chromosome 11q13 loss of heterozygosity (LOH) frequently occurs in the neoplasms of affected patients [15–17]. The present case of monozygotic twins with different MEN1 phenotypes suggests that a second hit may be independent of genetic background at least in the pancreatic lesion. Careful follow-up is required to detect other MEN1-related lesions in these interesting cases.

A variety of mutations (more than 80) have been reported in the MEN1 gene to date [4–7, 11]. The mutations include deletions, nonsense mutations, insertions, and missense mutations, of which the deletions are the most common mutations. They are scattered on the entire coding region of the MEN1 gene from exon 2 to 10, depending on the length of each exon. Exons 2 and 3 are the most common regions for the appearance of mutations probably due to their large size but this does not mean they are the true "hot spots" as reported in the RET gene of MEN2 [18]. A frameshift mutation (569del1, exon 3), identified in the present family appears to represent the most common type of MEN1 gene mutation. There is no direct evidence for the role of MEN1 gene mutations in the tumorigenesis of MEN1 due to the lack of information regarding the function

and structure of the MEN1 gene product, menin. Guru *et al.* recently reported that menin is a nuclear protein which has at least 2 independent nuclear localization signals (NLS) in the C-terminal portion [19]. They showed that the MEN1 gene mutations disrupting the NLS alter the nuclear localization of menin. In the present case, the deletion and frameshift mutation produced a stop codon at codon 184 in the MEN1 gene, resulting in menin truncation. The truncated menin without NLS may alter nuclear localization.

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