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Germline Mutations of the *MEN1* Gene in Japanese Kindred with Multiple Endocrine Neoplasia Type 1

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Multiple endocrine neoplasia type 1 (MEN1) is an autosomal dominant familial cancer syndrome. The responsible gene *MEN1* has recently been isolated, and its germline mutations have been identified in affected individuals in the United States, Canada and Europe. We screened for *MEN1* mutations by direct nucleotide sequencing of all protein-coding regions, and identified five distinct germline mutations in five among six Japanese kindreds with familial MEN1 or familial hyperparathyroidism. The mutations were dispersed across the gene. These findings suggest that, because of the absence of an obvious founder effect, the entire *MEN1* gene region should be examined for germline mutations in the probands of MEN1 and related syndromes in Japanese families.

Key words: Multiple endocrine neoplasia type 1 — *MEN1* — Familial cancer syndrome — Mutation — DNA testing

Multiple endocrine neoplasia type 1 (MEN1) is an autosomal dominant inherited disorder characterized by tumors of the parathyroids, enteropancreatic endocrine tissues, anterior pituitary and other tissues.[1,2] The responsible gene locus was mapped to chromosome 11q13 by linkage analysis and studies on loss of heterozygosity in MEN1-related tumors.[3,4] Very recently, Chandrasekharappa *et al.* identified the responsible gene, *MEN1*, which encodes menin, a 610-amino-acid protein of unknown physiological function.[5] *MEN1* is a putative tumor suppressor gene, and, according to Knudson's two-hit model, its heterozygous germline mutation underlies the inherited predisposition to tumorigenesis.[6]

Forty-seven different *MEN1* germline mutations have so far been identified in affected individuals in the United States and Canada[7] and in Europe.[8] Several identical mutations occurred in two or more kindreds not known to be related. Haplotype analysis suggested a founder effect for some, if not all, of these clusters of mutation.[7] *MEN1* gene mutations have not yet been investigated in Japanese families. To provide a basis for genetic diagnosis of MEN1 and its carriers in Japan, we examined *MEN1* germline mutations in Japanese kindreds of familial MEN1 and familial hyperparathyroidism. Because previous screening by dideoxy fingerprinting[9] could not detect mutations in some affected individuals,[5,7] we adopted a more reliable screening method, that is, direct sequencing of all protein-coding regions of the *MEN1* gene.

Blood or normal tissues were collected from eight affected individuals in six kindreds consisting of five kindreds of familial MEN1 (FMEN1) and a kindred of familial hyperparathyroidism (Table I). FMEN1 was defined as two or more of the three principal endocrine tumors of MEN1 with a family history of MEN1-related endocrinopathy. Familial hyperparathyroidism was defined as primary hyperparathyroidism in two or more family members without MEN1. The proband of family D (Table I) had multiple parathyroid hyperplasia without detectable abnormalities in the pituitary or in the pancreas, had a family history of surgically proven parathyroid adenomas in her mother and brother, and was diagnosed with familial hyperparathyroidism. Informed consent was obtained for a genetic study of all patients.

Genomic DNA was extracted from blood samples or other normal tissue with a QIAamp blood kit or QIAamp tissue kit (Qiagen, Hilden, Germany). All protein-coding regions of exons 2 through 10 of the *MEN1* gene (Fig. 1)

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Table I. Germline Mutations of the *MEN1* Gene

Family	Clinical diagnosis	*MEN1* mutation				
		Exon	Codon	Nucleotide change	Type of mutation	Abbreviation[a]
A	FMEN1	2	119	AAGAAG → AAG	in-frame deletion	K119del
B	FMEN1	2	125	ATATGG → ATGG	frameshift mutation	483del2
C	FMEN1	4	260	CAG → TAG	nonsense mutation	Q260X
D	Familial hyperparathyroidism	8	353	TAC → TAA	nonsense mutation	Y353X
E[b]	FMEN1	9	442	CAG → TAG	nonsense mutation	Q442X
F[b]	FMEN1	No mutation detected				

DNA was extracted from whole blood except for family A, in which it was prepared from the normal pancreatic tissue.
a) Mutation abbreviations follow standard nomenclature.[5,7,13]
b) Two affected individuals were examined.

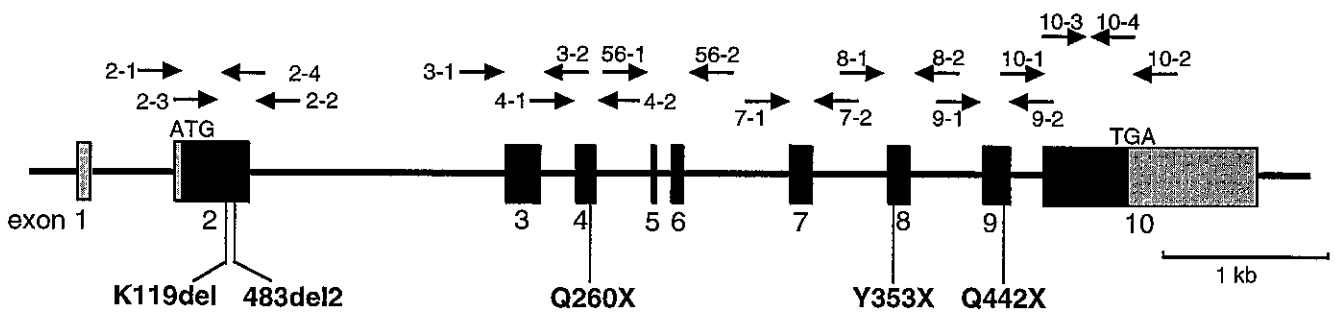

Fig. 1. Location of primers for PCR and nucleotide sequencing of the *MEN1* gene, and identified *MEN1* germline mutations. Protein coding and non-coding regions of the gene are shown by solid and shaded boxes, respectively. Introns and 5′ and 3′ flanking regions are shown by thin lines. Arrows indicate locations and orientations of the primers. Identified mutations are shown below. ATG, transcription start codon; TGA, termination codon.

Table II. Nucleotide Sequences and Locations of PCR Primers

Sense primer	Position[a]	Antisense primer	Position[a]
2-1: gggtggaaccttagcggaccctgg	2185–2208	2-4: cggccacgggaaagtaggtgaggc	2508–2531
2-3: gtcaaccgcgtcatccctaccaacg	2435–2459	2-2: tcatggataagattcccacctactggg	2740–2766
3-1: agggagtgtggcccatcactacctg	4213–4237	3-2: tcccttgggtggcttgggctactac	4544–4568
4-1: gtcattccctgaagcaggcacaggg	4651–4675	4-2: gggtcccacagcaagtcaagtctgg	4881–4905
56-1: aaggacccgttctcctccctgttcc	5121–5145	56-2: ggcccctgcctcagccactgttag	5421–5444
7-1: gggcatttgtgccagcagggcagc	5958–5981	7-2: cctggacgagggtggttggaaactg	6212–6236
8-1: tggggctacccccgatggtgagac	6562–6585	8-2: ctgtccaggtgggaggctggacac	6782–6805
9-1: gcctggcctgtgccctctgctaag	7126–7149	9-2: gctgctgtcaccacctgtagtgcc	7387–7410
10-1: tggccggcaaccttgctctcaccttt	7534–7558	10-2: ggtccccacaagcggtccgaagtc	8070–8093

a) Numbered according to Chandrasekharappa *et al.*[5] (GenBank accession number: U93237).

were amplified by polymerase chain reaction (PCR) in a 50 μl reaction mixture containing 150–250 ng of genomic DNA, one of nine pairs of primers (Table II) and Taq DNA polymerase (Takara Taq, Takara, Tokyo), using an automated thermal cycler (Robocycler Gradient, Stratagene, La Jolla, CA). PCR conditions were as follows: 1 min at 95°C for 1 cycle; 1 min at 95°C, 1 min at 64°C for exons 2 and 3, 66°C for exons 4, 5 and 6, 68°C for exons 7, 8, 9 and 10, and 2 min at 72°C for 30–31 cycles; 5 min at 72°C for 1 cycle. PCR products were purified with a QIAquick PCR purification kit (Qiagen). Nucleotide sequences were determined by direct se-

quencing of the PCR product with a DNA sequencing kit and an automated DNA sequencer (Dye Terminator Cycle Sequencing Ready Reaction and ABI PRISM310, Perkin Elmer, Foster City, CA). Both coding and noncoding strands were sequenced with PCR primers. Additional primers 10-3 (5′-ctcctgggactgtcgctggcacag-3′) and 10-4 (5′-cctgagccgtgctgccaccttcag-3′) were also used for the sequencing of exon 10 (Fig. 1). All identified mutations were confirmed by the cloning of the PCR products into pCRII vector (Invitrogen, Carlsbad, CA) and nucleotide sequencing.

Five different germline mutations were detected in five Japanese families, including four FMEN1 kindreds and a kindred of familial hyperparathyroidism, whereas no mutation was detected in a FMEN1 family, in which two affected individuals were examined (Fig. 1 and Table I). All mutations were confirmed to be heterozygous by identifying both normal and mutant clones of the PCR products. Individuals examined in the same kindred had identical mutations (family E). The mutations K119del and Q260X were identical with those reported previously,[7,8] while the other three mutations, 483del2, Y353X and Q442X, were different from all mutations described previously. The nonsense mutations, Q260X, Y353X and Q442X, and the frameshift mutation, 483del2, cause truncation of menin, which would most likely result in loss of function of the protein. Although the functional consequence of the in-frame deletion, K119del, is not easily deducible, this should not be a polymorphism, since the same mutation was described in two other MEN1 kindreds previously.[7] Therefore, we conclude that all these germline mutations cause a familial predisposition to tumorigenesis. A reported polymorphism, D418D (GAC → GAT),[7] was also observed in two kindreds.

Previous analysis of *MEN1* mutations in a large series of FMEN1 and related states involved dideoxy fingerprinting[9] for the initial screening, and encountered several FMEN1 kindreds, in whom no *MEN1* mutation was identified.[5,7] Therefore, we adopted direct sequencing of all protein-coding regions of the gene as the initial screening. This screening strategy would be less likely to miss mutations than dideoxy fingerprinting. Nevertheless, we could not detect *MEN1* germline mutations in individuals of a FMEN1 kindred, who would be expected to have a mutation in the gene. A similar mutation-negative FMEN1 has also been reported in Europe.[8] These kindreds may have had a mutation in the untranslated region, an intron or the promoter region of the gene, or a large germline deletion, which resulted in amplification of only the normal copy of *MEN1* by PCR.

A kindred of familial hyperparathyroidism (family D) had a germline mutation, Y353X. This is apparently contradictory to the previous results that no *MEN1* mutation was detectable in five kindreds of familial hyperparathyroidism.[7] Parathyroid tumors in MEN1 have the highest penetrance of all MEN1-related neoplasms, and are diagnosed as familial hyperparathyroidism before the development of other endocrinopathies.[1,2] On the other hand, a kindred of familial hyperparathyroidism was described previously, which had no linkage to the *MEN1* loci on chromosome 11.[10] Thus, there seem to be at least two types of familial hyperparathyroidism, a variant of FMEN1 and a distinct disease caused by another gene(s). Our patient is likely to belong to the former, and may eventually be diagnosed as having FMEN1.

The *MEN1* germline mutations in Japanese kindreds were all different among families, thus excluding an obvious founder effect, which has been identified in Newfoundland.[11] This is consistent with the previous findings in haplotype analysis of Japanese MEN1 families.[12] The *MEN1* mutations in Japanese kindreds spread across the gene. These findings emphasize the importance of examining the entire *MEN1* gene regions for DNA testing of MEN1 in Japanese probands. The direct sequencing of all protein-coding regions, which was employed in this study, is an efficient screening method for the detection of germline mutation, although the absence of a detectable mutation does not exclude the diagnosis of MEN1.

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