A Novel Six-nucleotide Insertion in Exon 4 of the MEN1 Gene, 878insCTGCAG, in Three Patients with Familial Insulinoma and Primary Hyperparathyroidism

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Three Japanese patients (a man and his two sons) in a family with clinical diagnosis of familial multiple endocrine neoplasia type 1 (MEN1) suffered from insulinoma(s), primary hyperparathyroidism and pituitary microadenoma. Genomic DNA of the patients was analyzed by sequencing for the MEN1 gene and an insertion of six nucleotides, CTGCAG, in exon 4, resulting in insertion of two amino acids, Leu–Gln, after the 256th amino acid of the menin (256insLQ), was identified. CTGCAG is a palindromic sequence and repeated twice in the wild-type allele (nucleotides 879–890). It is speculated that mutations involving only exon 4 of the MEN1 gene might induce development of insulinoma(s).

Key words: MEN1 – in-frame insertion – multiple endocrine neoplasia type 1 – familial insulinoma – hyperparathyroidism – menin

GENETIC SUMMARY

Disorder: Multiple endocrine neoplasia type 1
Ethnicity of patients: Japanese
Gene: MEN1
GenBank accession number: HSU93236, HSU93237
Chromosomal assignment: 11q13
Type of DNA variant: a germline in-frame insertion mutation
Mutation: 878insCTGCAG, insertion of six nucleotides, CTGCAG, in exon 4 of the MEN1 gene (after the 878th nucleotide in MEN1 cDNA), resulting in insertion of two amino acids, Leu–Gln, after the 256th amino acid (256insLQ)
Allelic frequency: 0/152 normal alleles
Method of mutation detection: PCR/direct sequencing
Data base searched: http://archive.uwcm.ac.uk/uwcm/mg/search/120173.html

CASE REPORT AND GENETIC ANALYSIS

Multiple endocrine neoplasia type 1 (MEN1) is an autosomal dominant disorder characterized by varying combinations of tumors involving the parathyroids, enteropancreatic neuroendocrine tissues and anterior pituitary. Germline mutation of the MEN1 gene has been reported in more than 80% of familial MEN1 (1–9) and about half of sporadic MEN1 (10). In contrast to MEN2, tumors producing various kinds of hormones may develop in the pituitary and pancreas in patients with MEN1, making the management of patients and mutant carriers difficult, because a genotype–phenotype correlation has not been established.

The proband Japanese patient III-6 (Fig. 1) had an operation for spinal ependymoma (C4-Th5) at the age of 51 years and
was found to have hypercalcemia (11.5 mg/dl). He was diagnosed as having primary hyperparathyroidism due to parathyroid hyperplasia. Four hyperplastic parathyroid glands were removed, one of which was autotransplanted. Subsequently, he had several hypoglycemic attacks due to insulinomas, which were completely removed by surgery. He also has a non-functioning pituitary microadenoma as detected by MRI of the head.

Patient IV-1 (Fig. 1), a son of the proband, had had repeated episodes of unconsciousness and generalized convulsion since he was 8 years old. An insulinoma with a size of 6 × 8 mm was detected at the pancreatic head, which was successfully

Figure 1. Pedigree of the patients’ family. E+ indicates the presence of the germline 878insCTGCAG by PCR/direct sequencing analysis.

Figure 2. Direct sequencing of the PCR product containing the exon 4 and 5' region of intron 4 of the \textit{MEN1} gene from a normal subject (upper panel) and the proband (III-6). The boxed and underlined CTGCAG sequence is the inserted and repeated one. Intronic sequences are designated with lower-case letters. Antisense sequence confirmed the same deletion (not shown). Two sons (IV-1 and IV-2) of the proband had the same mutation.
removed by surgery at the age of 9 years. Recently, he was found to have hypercalcemia (11.6 mg/dl) due to primary hyperparathyroidism and pituitary microadenoma secreting prolactin, although no further evaluation has been performed yet.

Patient IV-2 (Fig. 1), a younger brother of patient IV-1, had repeated hypoglycemic attacks and was operated on for multiple insulinomas located from the head to the tail of the pancreas at the age of 17 years. Primary hyperparathyroidism was diagnosed at the age of 24 years when he suffered from urolithiasis and hypercalcemia (11.3 mg/dl); enlargement of four parathyroid glands was noted by cervical echography and parathyroid scintigraphy. Non-functioning pituitary microadenoma was detected by MRI of the head.

The three patients were diagnosed as having familial MEN1. They had a common and characteristic feature of insulinoma(s), primary hyperparathyroidism and pituitary tumor. Further, as shown in Fig. 1, a cousin (III-5) of patient III-6 had died of insulinoma although detailed information was not available.

Nucleotide sequences of the exonic regions of the MEN1 gene from nucleotide 88 to 1988 covering the full-length coding region and those of intronic regions at exon–intron boundaries containing at least 38 nucleotides were determined. Further, as shown in Fig. 1, a cousin (III-5) of patient III-6 had died of insulinoma although detailed information was not available.

Nucleotide sequences of the exonic regions of the MEN1 gene from nucleotide 88 to 1988 covering the full-length coding region and those of intronic regions at exon–intron boundaries containing at least 38 nucleotides were determined in both orientations in the peripheral blood cells from the patients (9). A novel, heterozygous and germline mutation of the MEN1 gene in exon 4, 878insCTGCAG (insertion of six nucleotides CTGCAG after the 878th nucleotide in MEN1 cDNA) (Fig. 2), resulting in insertion of two amino acids, Leu–Gln, after the 256th amino acid of the menin (256insLQ), was identified in the three patients. The 878insCTGCAG is considered not to be a rare polymorphism but to be a pathologic mutation because the 878insCTGCAG was not found in 152 independent normal alleles and because sequences of amino acids 189–313 were completely identical among human, rat and mouse homologues (11). CTGCAG is a palindromic sequence and repeated twice in the wild-type allele (nucleotides 879–890) (Fig. 2). The mutant allele gained one more repeat, probably owing to a mechanism such as slippage error.

In this family, all patients had insulinoma(s) and III-5, a cousin of the proband III-6, died from insulinoma. A special predisposition to insulinoma is a characteristic feature of this family. A contribution of unknown modifier genes may be involved. Otherwise, this specific genotype of the MEN1 gene might be related to the specific phenotype. Out of our 72 MEN1 patients and family members with MEN1 germline mutations and well-defined clinical information, 14 cases developed insulinomas, 14 cases had MEN1 germline mutation involving only exon 4 (missense mutation or in-frame insertion or deletion in exon 4 and skipping of the entire 129 bp exon 4) and seven cases had both insulinoma(s) and exon 4 mutation (9,10,12). Chi-squared analysis (7/58 vs 7/14) showed a significant difference ($P = 0.0022$), suggesting a possible correlation between insulinoma development and mutations in exon 4 where Jun D binding occurs. However, most MEN1 cases with insulinoma were sporadic (eight cases) and only one family with insulinoma predisposition was detected in 46 familial MEN1 patients from 18 families.

**METHODS FOR MUTATION DETECTION**

PCR/direct sequencing of exon 4 and the franking introns was performed with the following conditions and parameters:

- **PCR primer, forward:** 5‘CCTGAACGGCAGCAAGGTG3’
- **PCR primer, reverse:** 5‘CTGCCAGGGTCCCCAGCAAA3’
- Size of PCR product: 256 bp
- **Thermal cycle profile:**
  - Initial denaturation: 94°C, 5 min
  - 35 cycles of 94°C, 60 s/58°C, 60 s/72°C, 60 s
  - Final extension: 72°C, 10 min
- **Sequencing primer:** the same as the PCR primers

PCR/direct sequencing of regions other than exon 4 was performed as previously described (9).

**References**