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Allele Loss on Chromosome 11 in a Pituitary Tumor from a Patient with Multiple Endocrine Neoplasia Type 1

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We have examined the allele loss of chromosome 11 in a pituitary tumor from a patient with familial multiple endocrine neoplasia type 1 (MEN 1). The extensive loss of chromosome 11, including loci of *D11S149*, *HRAS1* and *F2*, was detected by the loss of heterozygosity. All of the lost alleles of these loci were transmitted from the unaffected father and not from an affected mother. This is the first evidence of allele loss of chromosome 11 in a pituitary tumor of MEN 1 and supports the idea that similar allelic deletion of *MEN1* locus on chromosome 11 is the common genetic basis for tumorigenesis in the pituitary, endocrine pancreas, and parathyroid gland in MEN 1.

Key words: Allele loss — Chromosome 11 — Pituitary tumor — Multiple endocrine neoplasia type 1

Multiple endocrine neoplasia type 1 (MEN 1) is a familial disorder with an autosomal dominant inheritance in which tumors or hyperplasia occur in the pituitary, parathyroid, and endocrine pancreas.[1] The *MEN1* locus has been recently mapped to chromosome 11q13 by linkage analyses.[2–4] Allele loss on chromosome 11 as a molecular etiology for tumorigenesis has been confirmed only in the pancreatic and parathyroid tumors,[2, 5–11] but not in a pituitary tumor of MEN 1 examined.[10] The purpose of this study was to examine whether allele loss on chromosome 11 is found in a pituitary tumor of MEN 1 as well as the parathyroid and endocrine pancreatic tumors or not.

The patient was a 36-year-old female with a familial occurrence of MEN 1 (Fig. 1). From the age of 24, she had experienced hypoglycemic unconsciousness due to insulinoma, which was surgically removed. At the age of 36, she was admitted to our hospital for amenorrhea and galactorrhea. Biochemical studies revealed increased levels of plasma calcium, GH and prolactin, and her parathyroid and pituitary tumors were extirpated. Immunohistochemical examination of the pituitary adenoma indicated the presence of GH- and prolactin-producing cells. The screening test of relatives for MEN 1 showed that her mother had parathyroid tumors and a pituitary tumor.

The methods of DNA extraction from tumor specimens and leukocytes were the same as described previously.[5] DNA was completely digested with restriction endonuclease, then subjected to 0.7% agarose gel electrophoresis, and was transferred to a nylon membrane (Hybond-N, Amersham) after denaturation. Hybridization was carried out under conditions previously described.[5] The membranes were exposed for autoradiography on Kodak XAR films for 24–48 h. The probe "pTHH26" was used to detect the *D11S149* locus on chromosome 11, which has no recombination rate with the MEN 1 locus.[4] As shown in Fig. 2, the sizes of two *Pvu*II fragments are 5.2 (allele 1) and 3.2 kb (allele 2). The constitutional DNA from the leukocytes of this patient was heterozygous at this locus, but the allele 1 is lost in both the pituitary and parathyroid tumors. However, the loss in the parathyroid tumor was partial in the sense that the signal intensity of allele 1 was reduced to 23% at this locus. Unavoidable contamination of normal diploid tissue may well account for a small amount of allele 1. The constitutional DNA of the unaffected father and the affected mother of this patient was allele 1/2 and allele 2/2 for this locus, respectively. The lost allele is, therefore, transmitted from the unaffected father.

In addition to the loss of the paternally transmitted allele of *D11S149*, the loci of *HRAS1* (11p15.5) and *F2* (prothrombin) (11p11-q12) were also lost in the pituitary tumor. Loss of heterozygosity at these loci was analyzed in amplified DNAs by using the polymerase chain reaction (PCR)[12] to overcome the problem of the small amount of DNA available from the pituitary tumor obtained by the trans-sphenoidal surgical approach. Two approches were adopted. The first was to amplify genomic DNA by PCR and then to conduct endonuclease digestion. The second was to identify the heterozygosity from the size of PCR-amplified variable tandem repeats (VTR). Oligonucleotides used in this study were synthesized by an Applied Biosystems 392 DNA synthesizer (Applied Biosystems Japan, Tokyo). The sequences of the synthesized oligonucleotides were as follows: VTR

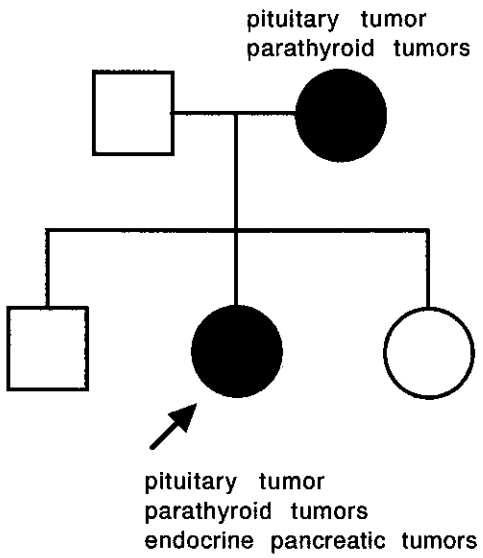

Fig. 1. The pedigree of a MEN 1 family. Females are represented by circles, and males by squares. Affected individuals are shown as filled symbols. An arrow indicates the proband.

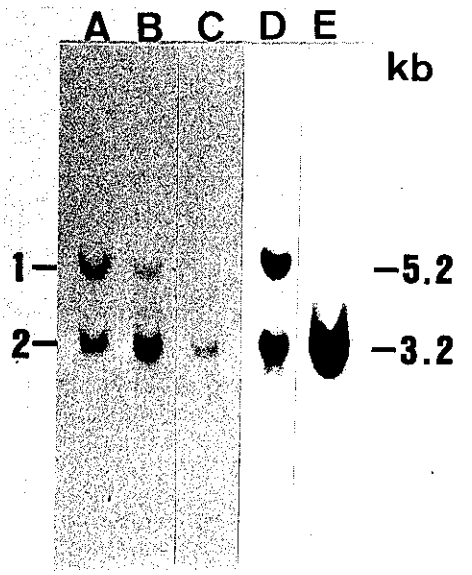

Fig. 2. Loss of heterozygosity at *D11S149* in the parathyroid and the pituitary tumors from a patient with familial multiple endocrine neoplasia type 1. DNAs of two tumors and peripheral leukocytes were obtained from a patient with MEN 1 and peripheral leukocytes of the father and the affected mother. DNAs were digested with *Pvu*II, separated by electrophoresis in a 0.7% agarose gel, and transferred to a nylon membrane. The Southern blot was hybridized with a ^{32}P-labeled pTHH26 probe. Lane A, DNA from peripheral leukocytes of a MEN 1 case; lanes B and C, DNAs from the parathyroid and pituitary tumors, respectively; lanes D and E, DNAs from peripheral leukocytes of the father and the affected mother, respectively. The numbers 1 and 2 on the left indicate the number allocated to each of the alleles to show the origin of the band.

region of the c-H-*ras 1* gene,[13] 5′-GGTTCCCTATCC CTGAGGTT-3′ and 3′-TAGCTATCTGAAGGGCTC-CGGTC-5′; exon 5 to 6 of the human prothrombin gene,[14] 5′-AATAAGTCCCCAGGCTCCAA-3′ and 3′-TCACCCCGCTGGGTACTGGT-5′. PCR was performed in a 10 μl reaction volume containing 0.1 μg of purified genomic DNA, 40 μM of each deoxynucleotide triphosphate with 370 kBq of [α-^{32}P]dCTP (111 TBq/mmol) in amplification of the c-H-*ras 1* gene or without radioactive dCTP in amplification of the prothrombin gene, 10 mM Tris-HCl, pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl, 0.001% gelatin, 10 pmol of each PCR primer and 0.25 unit of Taq DNA polymerase (Perkin-Elmer Cetus Corp., CT). The reaction was carried out using a Program Temperature Control System PC-700 (ASTEC, Fukuoka) with a temperature cycle of 1 min at 94°C, 1 min at 60°C and 2 min at 72°C. After 20 cycles, the amplified materials of c-H-*ras 1* gene were subjected to 5% polyacrylamide gel electrophoresis. The gels were dried and exposed for 1 h to Kodak XRP films. The amplified DNA of prothrombin gene after 30 cycles was phenol/chloroform-extracted and ethanol-precipitated. The DNAs were resuspended in TE buffer (10 mM Tris-HCl, pH 8.0,1 mM EDTA) and amplified DNAs were digested with *Nco*I, analyzed on an 8% polyacrylamide gel and visualized with ultraviolet light after staining with ethidium bromide. Substitution of T for C at position 4,203 of the human prothrombin gene[14] introduces an *Nco*I site and creates a new restriction fragment length polymorphism.[15] Owing to this polymorphism, the sizes of fragments obtained by *Nco*I cleavage are a constant fragment of 177 bp and either a fragment of 241 bp (allele 1) or fragments of 140 and 101 bp (allele 2). Figure 3A demonstrates that the size of the VTR region of c-H-*ras 1* gene in this patient was 820 (allele 1) and 750 bp (allele 2), which showed heterozygosity at this locus, and the loss of allele 2 was found in the pituitary tumor. In addition, loss of allele 2 at the *F2* (prothrombin) locus was found in the pituitary tumor (Fig. 3B). These results revealed an extensive range of loss on chromosome 11 and the loss of alleles at *HRAS1* and *F2* as well as *D11S149* in the pituitary tumor is on the counterpart of the chromosome containing the normal *MEN1* allele transmitted from her father. Allele loss at the loci of the *HRAS1* and *F2* in the parathyroid tumor was not analyzed by PCR, because of its admixture with normal cells as shown in Fig. 2. The loci of *PYGM, PGA* and *INT2* from 11q13, which are more tightly linked to the *MEN1* locus than *D11S149*,[2-4] were not informative in this patient due to homozygosity.

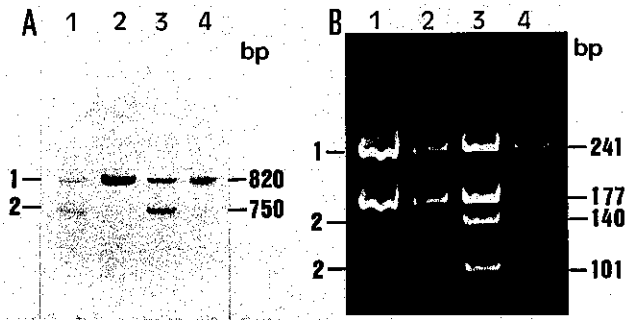

Fig. 3. Loss of heterozygosity at loci of *HRAS1* (A) and *F2* (B) in the pituitary tumor from a patient with familial multiple endocrine neoplasia type 1. DNAs were amplified by PCR using the appropriate primers for the VTR region of the c-H-*ras 1* gene (A) and exon 5 to 6 of the human prothrombin gene (B), separated by electrophoresis in a polyacrylamide gel, and autoradiographed (A) or stained with ethidium bromide (B). In A, DNAs from peripheral leukocytes from the father (lane 1), the affected mother (lane 2), a MEN 1 case (lane 3), and the pituitary tumor (lane 4) were analyzed. In B, DNA from peripheral leukocytes from the affected mother (lane 1), the father (lane 2), and a MEN 1 case (lane 3), and the pituitary tumor (lane 4) were analyzed. The numbers 1 and 2 on the left indicate the number allocated to each of the alleles to show the origin of the band.

No sequence alteration was detected by single-strand conformation analysis[16] at codon 12, 13, or 61 of the c-H-, K-, N-*ras* genes, at codon 201 or 227 of the α-chain of G_s gene, at codon 179 of the α-chain of G_{i2} gene or in exons 5, 6, 7 or 8 of the *p53* gene in the pituitary tumor (our unpublished data). This suggests that these genetic changes may not contribute to tumorigenesis of the pituitary tumor of MEN 1.

This case, to our knowledge, has provided the first example of allele loss on chromosome 11 in the pituitary tumor from a patient with MEN 1. This study, therefore, supports the concept that similar allele loss of *MEN1* locus on chromosome 11 is the genetic basis for tumors or hyperplasia in the pituitary and other multiple endocrine organs in MEN 1.

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