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The First Documentation of Li-Fraumeni Syndrome in Korea

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Li-Fraumeni syndrome(LFS) is an autosomal dominant disorder that predisposes individuals to multiple forms of cancer including breast cancer, soft tissue sarcoma, brain tumor, osteosarcoma, leukemia, and adrenocortical carcinoma. Recently, germ-line mutation of the p53 tumor suppressor gene has been implicated in this familial disorder.

We report a case of a 25-year old woman who presented with bilateral breast cancer and uterine leiomyoma. Her mother had died of early-onset bilateral breast cancer. And her younger sister had breast carcinoma as well, which was identified at the age of 22, indicating her strong familial history. To test for the presence of the p53 germ-line mutation, we analyzed the genomic DNA from the peripheral blood of the proband and her sister by PCR-SSCP analysis of exon 5 through exon 8 of the p53 gene. As a result, a p53 mutation in exon 7 was detected in an allele, and it was shared with her sister as the same pattern. Sequencing analysis determined the altered nucleotide at codon 248(CGG>TGG) which is one of the most frequent mutation sites related to LFS. Therefore, this patient has the most consistent characteristic features of LFS phenotype and it is believed that this case is the first report of a family with Li-Fraumeni syndrome carrying the p53 germ-line mutation in Korea.

The abbreviations used are : LFS, Li-Fraumeni Syndrome ; PCR, polymerase chain reaction ; SSCP, single strand conformation polymorphism.

Key Words : *Li-Fraumeni syndrome, Breast cancer, p53, Germ-line mutation, PCR-SSCP*

INTRODUCTION

In 1969, Li and Fraumeni observed several families with an aggregation of specific cancer types such as childhood sarcoma and breast cancer, and suggested a new familial cancer syndrome, Li-Fraumeni syndrome(LFS) (Li and Fraumeni, 1969). LFS is an autosomal dominantly inherited familial cancer syndrome characterized by a high susceptibility to diverse malignant tumors. The spectrum of cancers in the syndrome has been recognized to include breast cancer, soft tissue sarcoma, brain tumor, osteosarcoma, leukemia, and adrenocortical carcinoma. These diverse tumor types in family members characteristically develop at unusually early ages and multiple primary tumors are frequent(Malkin, 1993). To define

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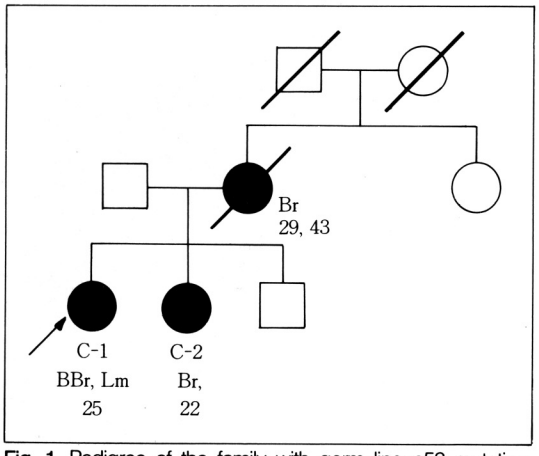

Fig. 1. Pedigree of the family with germ-line p53 mutation. □, male, normal ; ○, female, normal ; ●, female with cancer. Br, breast cancer ; BBr, bilateral breast cancer ; Lm, leiomyoma. Numbers under shape indicate ages at diagnosis while numbers after comma indicate age of death. The arrow marks proband and diagonal line, individual is dead.

the genetic background of LFS, several efforts have been made to search for the candidate gene of LFS. In 1990, inherited mutation within a defined region of the p53 tumor suppressor gene was suggested to underlie the defect in families with LFS(Malkin et al., 1990 ; Srivastava et al., 1990).

We recently identified a breast cancer prone family, in which cancers occurred at early ages. Subsequently, we also determined the presence of a germ-line mutation of the p53 tumor suppressor gene in the proband and her sister by PCR-SSCP and sequencing. It is thought that this case displaying characteristic features of LFS was the first documentation of Li-Fraumeni syndrome with germ-line mutation of the p53 gene in Korea.

CASE

A 25-year-old woman was admitted to Seoul National University Hospital due to palpable bilateral breast masses. One year before, she first noticed a left breast mass. The size of the mass increased slowly and 11 months later, she felt another mass in the right breast. There were left axillary masses as well. Her mother had suffered from breast cancer at age 29 and died of the disease at age 43(Fig. 1). Her younger sister had also had unilateral breast cancer at the age of 22 and had received a modified radical mastectomy followed by adjuvant chemotherapy at another hospital. The patient was single and denied other illnesses. On examination, both breast masses were nontender, hard, and movable. The size of the right and left masses were about 2cm×2cm and 9cm×7cm, respectively. Two left axillary masses were hard and fixed, and 1cm×1cm in size. Abdominal palpation and pelvis ultrasonography suggested a large pelvic mass. Bone scintigraphy revealed no bony abnormality. She underwent bilateral modified radical mastectomy and total abdominal hysterectomy with bilateral salphingoophorectomy. Pathologic examination showed infiltrating ductal carcinoma of bilateral breasts with metastasis to the left axillary lymph nodes and uterine leiomyoma.

MATERIALS AND METHODS

1. Tissue Samples and DNA Extraction

Blood samples were obtained from the proband, her sister, and two other young sarcoma patients with family history. Tested individuals in this study are listed in Table 1. The proband's primary tissues and lymph nodes were also obtained at the time of surgery as follows : both breast carcinomas, both axillary lymph nodes, both ovaries, and uterus. Fresh tissues were immediately frozen in liquid nitrogen and stored

Table 1. List of tested samples

No.	Key	Sex/Age	Cancer Types	Comments
1	Wt	M/25	normal	
2	a	F/17	chondrosarcoma	
3	b	F/15	osteosarcoma	
4	c-1	F/25	breast carcinoma	proband
5	c-2	F/22	breast carcinoma	proband's sister
6	KG-1		leukemic cell line	p53 point mutation in exon 6
7	HL-60		leukemic cell line	partial deletion in the p53

Table 2. The oligonucleotide primers used in PCR

exon	PCR products(bp)	primer sequence
exon 5	325	5′-TTCCTCTTCCTGCAGTACTC-3′
		5′-GCAAATTTCCTTCCACTCGG-3′
exon 6	236	5′-ACCATGAGCGCTGCTCAGAT-3′
		5′-AGTTGCAAACCAGACCTCAG-3′
exon 7	139	5′-GTGTTGTCTCCTAGGTTGGC-3′
		5′-CAAGTGGCTCCTGACCTGGA-3′
exon 8	330	5′-CCTATCCTGAGTAGTGGTAA-3′
		5′-CCAAGACTTAGTACCTGAAG-3′

at -70℃. High molecular weight DNA was isolated from blood and frozen tissues according to the standard protocol(Sambrook et al., 1989). As positive controls for p53 gene mutation, the DNA samples from KG-1 and HL-60, leukemic cell lines, were also used.

2. PCR-SSCP Analysis of p53

Portions of the p53 gene including exons 5 to 8 were amplified by polymerase chain reaction. The oligonucleotide primers used for amplification are shown in Table 2. Each amplification reaction was carried out in a 25 μℓ reaction volume containing 200 ng of template DNA, 10mM Tris-HCl(pH8.3), 1.5mM MgCl2, 50mM KCl, 10 pmol of each primer, deoxynucleoside triphosphates dATP, dGTP, dTTP, and dCTP at 200 μM each, 1 μCi of [α-P^{32}] dCTP(3,000 Ci/mmol), and 1 unit of Taq polymerase(Boehringer Mannheim, Germany). Reaction mixtures were subjected to 35 cycles of which each cycle consisted of denaturation at 95 ℃ for 40 sec, annealing at 55 ℃ or 60 ℃(for exon 7) for 1 min, followed by polymerization at 72 ℃ for 1 min. The PCR products were diluted 1 : 24 in loading solution(95% formamide, 10mM EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol), denatured at 95 ℃ for 5 min and loaded onto 6% non-denaturing polyacrylamide gel with 10% glycerol. Samples were electrophoresed at room temperature for 3-4 hr at 30W(Orita et al., 1989). Alternative gel condition used for confirmative detection of gene mutation was 50% MDE gel(AT Biochem, Inc., Malvern, PA, US) with 0.6X TBE. The samples were run as described above except for the voltage condition of 20 V/cm. Gels were transferred to 3MM Whatman paper, dried and autoradiographed with X-ray film at -70℃ for 4-18 hr.

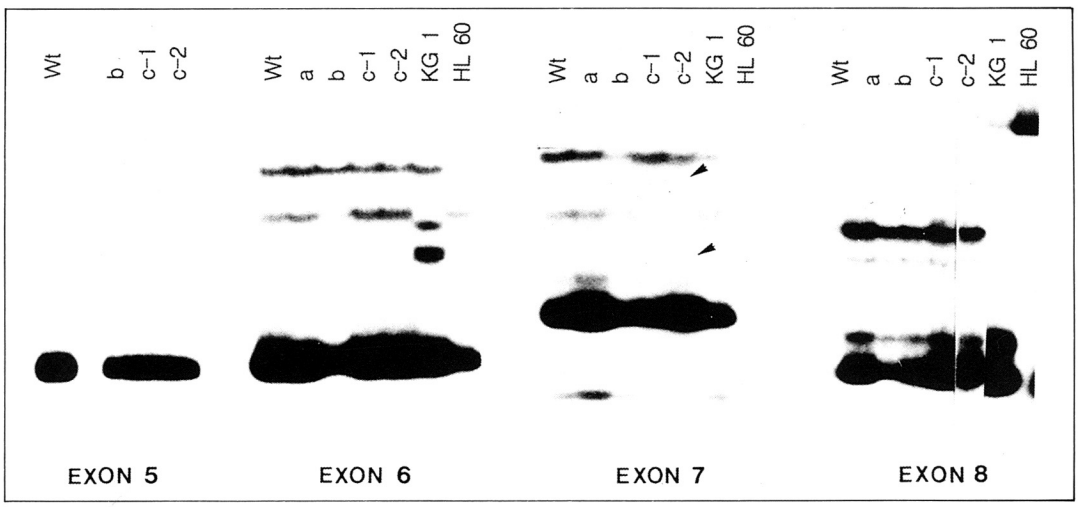

Fig. 2. PCR-SSCP analysis of p53 exon 5-8. Samples of c-1 and c-2 showed additional mobility shifted bands in exon 7. No mutation could be found in other exons.

3. Sequencing Analysis

To confirm the SSCP results and determine the altered nucleotide sequence, the samples that were identified as mutants were reamplified by PCR and purified. The products were then subcloned into ddT-tailed vectors prepared as previously described(Holton and Graham, 1990). Sequencing analysis was performed using Sequenase kit(United States Biochemical, Cleveland, Ohio, US).

RESULTS

1. PCR-SSCP Analysis

We initially analyzed DNA samples from peripheral blood. On electrophoresis, mobility shifted bands were detected in the exon 7 of p53 in the samples from the proband and her sister. There was no mobility shift in the other exons. The DNAs from the other patients showed no mobility shift in the exons tests(Fig. 2). The samples with altered mobility band showed additional bands with normal mobility, suggesting the single allele germ-line mutation. This finding was confirmed with alternative gel condition(Fig. 3). We next examined DNA samples from various surgical specimens from the proband. Mobility shifted bands for exon 7 were present in all the tissues tested, which showed the same pattern as peripheral blood(Fig. 4).

2. Sequencing Analysis

Sequencing analysis for two DNA samples with altered mobility showed the base change of C to T at the first position in codon 248 of exon 7(Fig. 5). The resulting amino acid transition was arginine to tryptophan, which is one of the most common p53 mutations in LFS families.

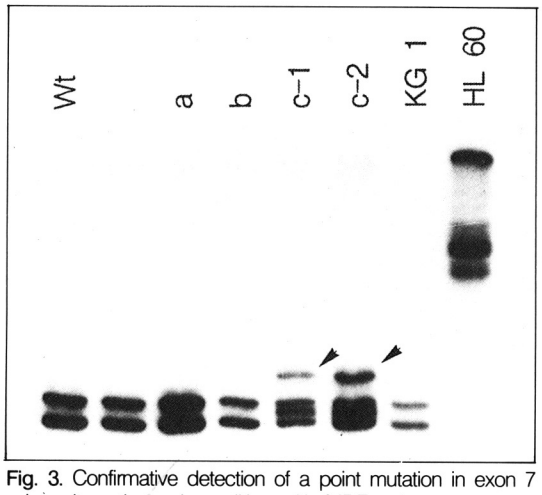

Fig. 3. Confirmative detection of a point mutation in exon 7 using alternative gel condition with MDE gel.

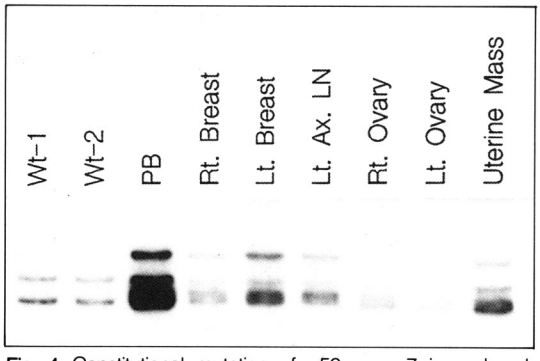

Fig. 4. Constitutional mutation of p53 exon 7 in proband. DNA samples of various tissues from proband showed the same genetic defects. PB, peripheral blood ; Rt. Breast, right breast ; Lt. Breast, left breast ; Lt. Ax. LN, masses at left axillary lymph node ; Uterine mass, uterine leiomyoma.

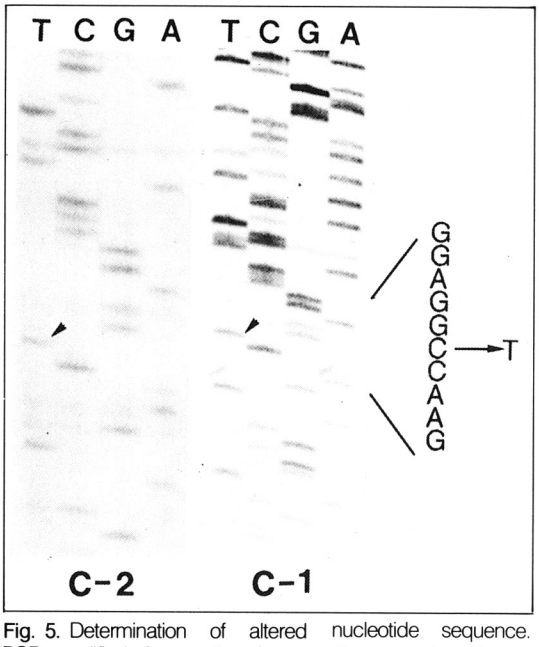

Fig. 5. Determination of altered nucleotide sequence. PCR-amplified fragments of exon 7 were cloned onto ddT-tailed vectors. Three colonies per individual were selected and sequenced. A missense mutation (CGG>TGG) at codon 248 is observed in proband (c-1) and her sister(c-2).

DISCUSSION

Li-Fraumeni syndrome is a clinical diagnosis based on the aggregation of diverse forms of cancer including breast cancer in a proband, and two or more close relatives. Approximately 50% of cancers in reported Li-Fraumeni families occur before 30 years of age. In young adults, premenopausal breast cancer is, by far, the most common neoplasm whereas soft tissue sarcoma and osteosarcoma are prevalent in children and in adolescence, respectively(Li et al., 1992). In our case, three members of the family had early-onset breast cancer(Fig.1) and presented a history that was consistent with LFS.

Human breast cancer occurs usually as a result of somatic mutation. However, hereditary breast cancer occurs and accounts for approximately 10% of all of breast cancer(Anderson, 1992). Four inherited types of breast cancer have been identified. Among them, LFS involves early-onset of breast cancer, bilaterality, and associated tumor in the family such as soft tissue sarcoma(Borresen, 1992).

Recently, germ-line mutations of the p53 gene have been found in affected members and obligate carriers in families with LFS(Malkin et al., 1990 ; Srivastava et al., 1990) indicating that the p53 germ-line mutation is the genetic basis for the LFS. The p53 tumor suppressor gene is located on the short arm of chromosome 17, band p13.1 and alterations of the gene are the most frequently encountered genetic events so far both in sporadic and hereditary human malignancies(Sager, 1989). The germ-line mutation of p53 seems to be distinct from the somatic mutation in that it occurs in a more defined region(between codon 245 and 258), which is within conserved region IV of the p53 gene. The mutations of this region are believed to functionally inactivate p53 protein and thereby provide an increased risk for cancer(Malkin et al., 1990 ; Frebourg et al., 1992 ; Malkin, 1993). It has become clearer that not all families with classic LFS have detectable germ-line mutations of the p53 gene and germ-line p53 mutation is not the sine qua non of LFS. However, little is known about the frequency of germ-line p53 mutations in hereditary breast cancer patients outside families with LFS. Although one study found 1 of 126 early-onset breast cancer patients to have carried mutant p53(Sidransky et al., 1992), most studies failed to find the germ-line mutation without LFS phenotype-(Malkin, 1993). In addition, the mutation of the carrier without LFS turned out to occur outside the conserved region and thereby it might be functionally silent(Frebourg et al., 1992). These results suggested that germ-line p53 mutations occur rarely in early-onset breast cancer outside the LFS(Borresen et al., 1992 ; Sidransky et al., 1992).

In this study, the PCR-SSCP result for lymphocyte DNA of the proband and her sister indicated that the family carry the germ-line mutation of the p53 gene-(Fig. 2 and 3). Mobility shifted bands were also detected in all the samples including peripheral blood, tumor tissues, and benign tissues as the same pattern, confirming that it is constitutional mutation(Fig. 4). Sequencing analysis showed that the p53 mutation is C to T change at the CpG dinucleotide moiety of codon 248 in exon 7, which leads to substitute amino acid arginine for tryptophan. This mutation is the most common p53 gene alteration in not only sporadic tumors but also hereditary tumors. And this site was previously suggested to be a mutational 'hot spot' in LFS(Santibanez et al., 1991 ; Eng and Ponder, 1993 ; Birch et al., 1994). This mutation is believed to have functional significance.

The family in our case had the most consistent characteristic features of LFS phenotype. It is believed that this is the first report of a family with Li-Fraumeni syndrome carrying the p53 germ-line mutation in Korea. In addition, our observations further confirm that this inherited p53 mutation may predispose members of the LFS family to increased susceptibility to cancer. However, it is not the time of occurrence of mutations, but rather accumulations, that is the most important in tumor development. In Li-Fraumeni syndrome, it is thought that p53 gene mutations occur first and other mutations follow(Vogelstein, 1990). Therefore, further studies regarding the subsequent genetic defects and its biological significance should be assessed.

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