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SHORT COMMUNICATION

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Germline mutations of *E-cadherin* gene in Korean familial gastric cancer patients

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Abstract Gastric cancer is the most common cancer in Korea. Germline mutations of the *E-cadherin* gene have recently been identified in familial gastric cancer patients. We screened five Korean familial gastric cancer patients to investigate germline mutations of the *E-cadherin* gene. These patients fulfilled the following criteria: presence of at least two gastric cancer patients within first-degree relatives and one patient diagnosed before the age of 50 years. Abnormal band patterns were found in exons 6 and 10 in two familial gastric cancer patients by polymerase chain reaction-single strand conformation polymorphism analysis (probands from the SNU-G2 and SNU-G1001 families, respectively). DNA sequencing analysis of the *E-cadherin* gene of these two patients revealed missense mutations in each exon. The SNU-G2 proband harbored a missense mutation from aspartic acid (GAT) to glycine (GGT) at codon 244 in exon 6 of the *E-cadherin* gene, and the SNU-G1001 proband had a missense mutation from valine (GTG) to alanine (GCG) at codon 487 in exon 10. The SNU-G2 proband was diagnosed with gastric cancer at the age of 38; three brothers and two sisters had died of gastric cancer under the age of 50, and their mother had died of gastric cancer at the age of 63. The SNU-G1001 proband was diagnosed with gastric cancer at the age of 42 and one brother had died of gastric cancer at the age of 49. In summary, we found germline mutations of the *E-cadherin* gene in two of five Korean familial gastric cancer patients screened.

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Introduction

Gastric cancer is a major cause of cancer death worldwide, and is the most common form of cancer in Korea. Epidemiological studies have shown that some cases represent familial aggregation (La Vecchia et al. 1992). Since the carcinogenesis of gastric cancer has not been clearly defined, there is some doubt in regard to the inherited gene alterations and environmental factors shared by family members (Shinmura et al. 1998).

E (epithelial)-cadherin is a member of the family of transmembrane glycoproteins that are responsible for calcium-dependent cell-to-cell adhesion. It is reported that E-cadherin is inactivated by irreversible genetic alteration in carcinomas of gastric origin, and the expression of E-cadherin is reduced in 45.5% of cancers of various organs, including stomach cancer (Shiozaki et al. 1996).

Recently, germline mutations of the *E-cadherin* gene were identified in familial gastric cancer patients of three kindreds from New Zealand (Guilford et al. 1998); this represents a clear molecular basis for familial gastric cancer. Germline mutations of the *E-cadherin* gene were also reported in families of European origin, especially in a high proportion of diffuse-type gastric cancer patients (Gayther et al. 1998). We therefore screened for the presence of germline mutations of the *E-cadherin* gene in five Korean familial gastric cancer patients.

Patients and Methods

Patients

We collected blood samples from a total of five Korean gastric cancer patients who had been treated at Seoul National University Hospital. These patients fulfilled the fol-

Table 1 Germline mutations of the E-cadherin gene in gastric cancer families

Family	No. of gastric cancer patients	Age (years) mean(range)	Age of proband (year)	Mutation of E-cadherin gene in proband				Histologic type
				Exon	codon	n.t. change	a.a. change	
SNU-G1	5	33 (22-57)	57	Wild type				Diffuse
SNU-G2	7	42 (30-63)	38	6	244	GAT→GGT	Asp→Gly	Diffuse
SNU-G1001	2	46 (42-49)	42	10	487	GTG→GCG	Val→Ala	Diffuse
SNU-G1003	2	36 (26-45)	26	Wild type				Diffuse
SNU-G1005	3	46 (33-56)	33	Wild type				Diffuse

n.t., Nucleotide; a.a., aminoacid

lowing criteria: presence of at least two gastric cancer patients within first-degree relatives and at least one patient diagnosed before the age of 50 years. All five patients examined were diagnosed with diffuse-type gastric carcinoma. Mutational analysis of the *E-cadherin* gene was performed with DNA samples from all five patients. For a genetic polymorphism study, we screened DNA samples from peripheral blood lymphocytes of 50 healthy persons and normal colonic mucosa DNA of 50 colorectal cancer patients.

DNA extraction

Peripheral blood lymphocytes of the gastric cancer patients and healthy persons were isolated using Ficoll-Paque according to the manufacturer's instructions (Pharmacia Biotech, Uppsala, Sweden). Total genomic DNA was extracted using TRI reagent, also according to the manufacturer's instructions (Molecular Research Center, Cincinnati, OH, USA).

Polymerase chain reaction (PCR) amplification and single strand conformation polymorphism (SSCP) analysis

To investigate the genetic alteration of the *E-cadherin* gene, we screened 16 exons by PCR-SSCP in the five Korean gastric cancer patients. The PCR primer pairs were prepared as described by Berx et al. (1995a). Non-radioactive PCR reactions were carried out in a volume of 25 μl containing 100 ng genomic DNA, 2.5 pmoles of each primer, four dNTP at 250 μM each, 0.5 units of Taq polymerase, and the reaction buffer provided by the supplier (Boehringer Mannheim, Mannheim, Germany). Reactions were initiated by denaturation for 5 min at 94°C and carried out for 35 cycles in a programmable thermal cycler (Perkin Elmer Cetus 9600; Roche Molecular Systems, Nutley, NJ, USA). PCR conditions consisted of 35 cycles of 94°C for 30 s, 55-60°C for 1 min, and 72 °C for 1 min, with a final elongation of 10 min at 72 °C.

For SSCP, the genomic DNA in each exon of the *E-cadherin* gene was amplified in a final volume of 10μl, using the same PCR procedure as that described above, but with the addition of [α-^{32}P]-dCTP (Amersham, Arlington Heights, IL, USA). The region from exons 4 to 5 was amplified in a single PCR. Radiolabeled PCR reaction products were mixed with 95% formamide dye, denatured at 94°C for 5 min, and chilled on ice. Three microliters of mixture was loaded on a nondenaturing SSCP gel, 6% polyacrylamide (19:1) with 10% glycerol in 1 × Tris Borate-EDTA (TBE) buffer, and separated for 12-16 h in a cold room (4 °C) at a constant 300 V. After electrophoresis, the gel was transferred to 3 MM Whatman paper, dried on a gel dryer and sumitted to autoradiography.

Cloning and sequencing

Samples showing abnormal bands by SSCP were submitted to cloning for DNA sequencing analysis. Fresh PCR products were ligated into pCR-TOPO vectors (product of Invitrogen company) and subcloned using the TA cloning system (Invitrogen, San Diego, CA, USA). A minimum of ten individual colonies were taken and cultured overnight in Luria-Bertani (LB) medium containing 50 μg/ml ampicillin. Plasmid DNA was isolated and used for DNA sequencing analysis. Bidirectional sequencing analysis was performed using the Taq dideoxy terminator cycle sequencing kit on an ABI 377 DNA sequencer (Perkin-Elmer, Foster city, CA, USA). Sequences of target DNA were determined by using the original PCR primers.

Results

All five patients examined were affected by diffuse type gastric cancers (Table 1).

To investigate the germline mutation of the *E-cadherin* gene, we screened 16 exons by PCR-SSCP in five Korean familial gastric cancer patients. From our PCR-SSCP analysis, abnormal band patterns were found in probands SNU-G2 and SNU-G1001 of each family in exons 6 and 10, respectively. However, there were no abnormal bands in the other samples including the five unaffected members of the SNU-G1 proband's family. DNA sequencing analysis of the *E-cadherin* gene in these two patients revealed missense mutations in each exon. The SNU-G2 proband harbored a missense mutation from aspartic acid (GAT) to glycine (GGT) at codon 244 in exon 6 of the *E-cadherin* gene (Fig. 1 and Fig. 2). The SNU-G1001 proband had a missense mutation from valine (GTG) to alanine (GCG) at codon 487 in exon 10. Reliability of the analyses was confirmed by PCR-SSCP in the 50 blood DNA samples of non-cancer patients and the 50 normal colonic mucosa DNA samples of colorectal cancer patients. The SNU-G2 proband was diag-

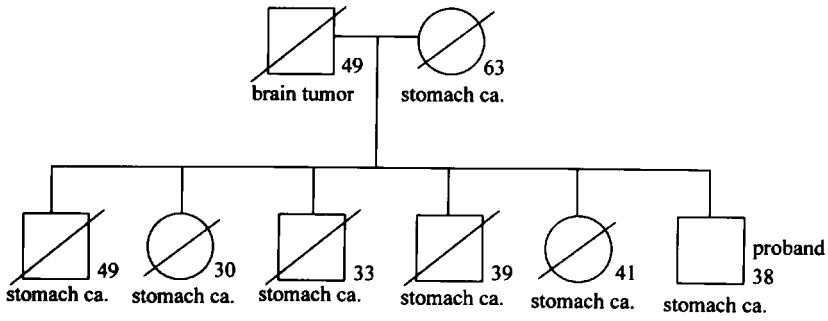

Fig. 1. Pedigree of SNU-G2 familial gastric cancer family. Individuals' ages are indicated to the *right of the symbols*. *Symbols*, *squares*, males; *circles*, females; *all symbols with a diagonal*, deceased; *Ca*, Cancer

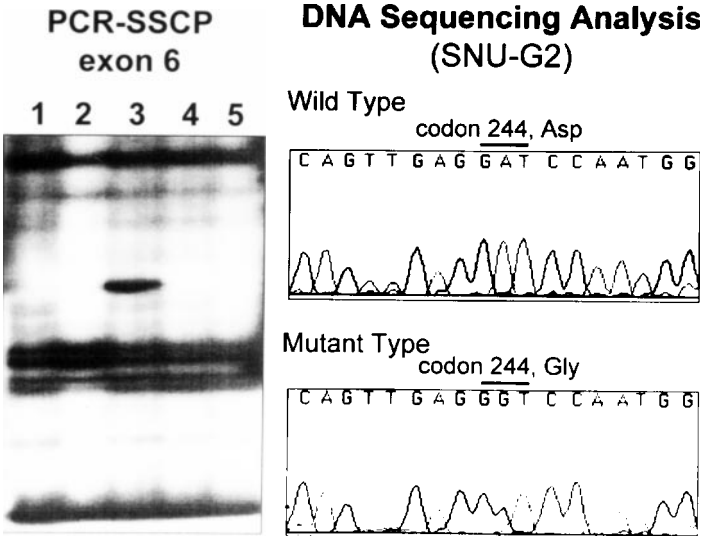

Fig. 2. Germline mutation of exon 6 in the *E-cadherin* gene of proband SNU-G2. As a result of polymerase chain reaction-single-strand conformation polymorphism (*PCR-SSCP*) analysis, abnormal band patterns were found in exon 6 of the SNU-G2 proband. *Lane numbers (1-5)* indicate each proband are; *lane 1*, SNU-G1; *lane 2*, SNU-G1001; *lane 3*, SNU-G2; *lane 4*, SNU-G1003; *lane 5*, SNU-G1005. DNA sequencing analysis of exon 6 of the *E-cadherin* gene revealed a missense mutation from aspartic acid (GAT) to glycine (GGT) at codon 244

nosed with gastric cancer at the age of 38 and his mother had died of gastric cancer at the age of 63 (Fig. 1). The SNU-G1001 proband was diagnosed with gastric cancer at the age of 42 and one brother had died of gastric cancer at the age of 49.

Discussion

The role of the *E-cadherin* gene in the tumorigenesis of gastric cancer has been established previously with somatic mutations in diffuse type gastric cancers (reviewed by Gayther et al. 1998). Becker et al. (1994) provided strong in-vivo evidence that *E-cadherin* gene mutations may contribute to the development of diffusely growing gastric carcinomas. Moreover, germline mutations of the *E-cadherin* gene have only been reported in familial gastric cancer patients with diffuse type gastric cancers (Guilford et al. 1998; Gayther et al. 1998). All five patients we examined were diagnosed with diffuse type gastric cancer.

We screened germline mutations of the *E-cadherin* gene in five Korean familial gastric cancer patients by PCR-SSCP and sequencing analysis. Missense germline mutations of the *E-cadherin* gene were detected in two patients. These two patients with germline mutations had been diagnosed with gastric cancer at the ages of 38 and 42, respectively, and both patients had a family history of gastric cancers.

Missense mutation of the *E-cadherin* gene is relatively rare compared with other types of mutation, including frameshift mutation (reviewed by Berx et al. 1998). Six truncating mutations and one missense mutation in the germline in familial gastric cancer patients have been reported in the literature (Guilford et al. 1998; Gayther et al. 1998). To date, only 11 missense mutations have been reported in various cancers. Of these, one was a germline mutation and the rest were somatic mutations. Moreover, 6 of these 11 missense mutations were identified in gastric cancers (reviewed by Berx et al. 1998). Our data showed two germline mutations at exons 6 and 10, respectively. To date, a total of 18 polymorphism sequence variants have been identified (reviewed by Berx et al. 1998). The two missense mutations identified in our study did not belong to these polymorphisms. Since these nucleotide changes were not detected in the 100 normal control DNA samples at exons 6 and 10, it is likely that these variants are germline mutations rather than genetic polymorphism. In addition, the two missense mutations occurred in highly conserved amino acid residues (Berx et al. 1995b) belonging to extracellular domains in Ca^{2+}-binding motifs, although the effect of these mutations on protein function should still be examined. In summary, we have presented

the identification and characterization of germline mutations of the *E-cadherin* gene in two of five Korean familial gastric cancer patients.

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