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SHORT COMMUNICATION

Familial gastric cancer: clinicopathological characteristics, RER phenotype and germline *p53* and *E-cadherin* mutations

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Gastric cancer frequently occurs in family members with hereditary non-polyposis colorectal cancer (HNPCC) and Li–Fraumeni syndrome (LFS) and germline *E-cadherin* mutations were recently identified in a subset of familial gastric cancers. Thus, families with an aggregation of gastric cancers were recruited by reviewing the genealogical trees of 3632 patients with gastric cancer. The criteria for recruiting such families were the following: at least three relatives should have gastric cancer and one of them should be a first degree relative of the other two; at least two successive generations should be affected; in one of the relatives gastric cancer should be diagnosed before age 50. Thirty-one cases (0.9%) fitted all three of these criteria. There were only gastric cancer patients in 18 of the 31 families and there were no families that fitted clinical criteria of HNPCC or LFS. Paraffin-embedded tissues were available in 29 probands and DNA was successfully isolated for molecular analyses in 13 probands. RER phenotype was detected in three (23%) cases, whereas germline *p53* mutations were detected in none of 13 cases. A germline *E-cadherin* mutation was detected in one of three diffuse types and none of 10 intestinal types, however, a mutation resulting in the replacement of Gly by Val was detected in the precursor sequence. Thus, although familial clustering of gastric cancer occurs in ~1% of gastric cancer patients, germline mutations of the DNA mismatch repair, *p53* and *E-cadherin* genes do not significantly contribute to such a clustering.

Gastric cancer is the second most common cancer in the world following lung cancer (1). As with colon, breast and several other cancers in which familial clustering has been reported, a high incidence of gastric cancer in close relatives of affected individuals has been reported (2–6). Thus, it has been suggested that genetic factors as well as environmental factors are important for the pathogenesis of gastric cancers (7–10). To evaluate the significance of inherited disorders for the development of gastric cancer, it is of great importance to recruit and characterize families with an aggregation of gastric cancer. In particular, gastric cancer often occurs in family members with hereditary non-polyposis colorectal cancer (HNPCC) (11) and Li–Fraumeni syndrome (LFS) (12–14). However, it is still debatable whether or not the replication error (RER) phenotype is associated with gastric cancer with familial aggregation (15,16) and there is no report on systematic germline mutation analysis of the *p53* gene in familial gastric cancers. It was recently reported that the *E-cadherin* gene, which is frequently mutated in sporadic diffuse type gastric cancer (17–19), is responsible for the inherited susceptibility to gastric cancer of the diffuse type (20,21). Thus, it is worth investigating the prevalence of an inherited disorder of the *E-cadherin* gene in gastric cancer families.

In several hereditary cancer syndromes, such as HNPCC, the mode of genetic transmission is consistent with an autosomal dominant inheritance pattern. In this study, we attempted to recruit hereditary gastric cancer cases with autosomal dominant inheritance patterns. Thus, familial cases were selected by criteria in accordance with the Amsterdam criteria for HNPCC as a model. In analogy to the diagnosis of HNPCC (Amsterdam criteria) (22), we tested the following criteria: (i) at least three relatives should have gastric cancer and one of them should be a first degree relative of the other two; (ii) at least two successive generations should be affected; (iii) in one of the relatives, gastric cancer should be diagnosed before age 50.

Probands of pathologically verified primary gastric cancer were identified from hospital records of patients who had been diagnosed between 1962 and 1995 at the National Cancer Center Hospital, Tokyo, Japan. Family histories were systematically obtained from patients and/or their family members at the time of hospitalization. Eligibility for this study was limited to probands whose family histories of the first and second degree relatives were available. In ~10% of cases, family histories of the patients were inadequate. Of the cases, 3632 conformed to these criteria. The ages of 3632 probands at diagnoses ranged from 16 to 89 years old (16–87 in male and 21–89 in female) and the mean age ± SD of the probands was 56.3 ± 12.1 years (56.9 ± 12.0 in male and 55.2 ± 12.4 in female). Information about cancer in the first and second degree relatives was collected retrospectively by standardized, written questionnaires given to the patients or members of their families.

The number of gastric cancer patients per family ranged from one to five and in 124 of the 3632 families (3.4%) at least three relatives had gastric cancer (Table I). Gastric cancer was diagnosed before the age of 50 in at least one of the relatives in 1037 of 3632 families (28.6%). Among these cases, 46 families fitted both criteria (i) and (iii). However, only one generation was affected by gastric cancer in seven of the 46 families. Also, in eight families with three gastric cancer patients, parents of the probands were affected by gastric cancer, thus we considered that there were only two relatives with gastric cancer in such families. After those 15 cases were excluded, 31 of 3632 families (0.9%) met all three of the criteria.

Abbreviations: HNPCC, hereditary non-polyposis colorectal cancer; LFS, Li–Fraumeni syndrome; RER, replication error; SSCP, single-strand conformation polymorphism.

Table I. Number of families meeting the criteria for familial aggregation of gastric cancer

Criteria	No. of families (%)
No. of gastric cancer patients per family	
⩽2	3508 (96.6)
⩾3	124 (3.4)
Age of the youngest gastric cancer patient	
<50	1037 (28.6)
⩾50	2595 (71.4)
Familial gastric cancer	
Did not meet the criteria	3601 (99.1)
Met the criteria	31 (0.9)

Table II. Characteristics of 31 families meeting the criteria for familial gastric cancer

Characteristics	No. of families
No. of gastric cancer patients	
3	20
4	7
5	3
6	1
Generations affected by gastric cancer	
2	28
3	3
No. of cancer patients	
3	14
4	8
5	6
6	2
7	1
Site of tumors	
Stomach only	18
Other organ involved	13
Lung	5
Uterus	3
Colorectum	2
Liver	2
Breast	1
Esophagus	1
Kidney	1
Larynx	1
Blood	1
Patients with multiple primary tumors	
One primary tumor	29
Two or more primary tumors	2
Histological subtype[a]	
Intestinal	17[b]
Diffuse	12[b]

[a]According to Lauren's criteria.
[b]Twenty-nine cases were available for pathological examination.

We next examined the detailed family histories of these 31 individuals (Table II). Pedigrees of the 31 families are shown in Figure 1. In 11 of the 31 families, more than four individuals were affected by gastric cancer and in three families, three successive generations were affected by gastric cancer. The number of cancer patients in each family ranged from three to seven. In 18 of 31 families, there were gastric cancer patients only and no patients with other types of cancer. In the remaining 13 families, there were 16 patients with other types of cancer. There were five patients with lung cancer, three patients with uterine cancer, two patients with colorectal cancer and two patients with liver cancer.

Breast cancer and leukemia are the component tumors in LFS and multiple primary cancer is one of the criteria for LFS (23). There was a patient with breast cancer in family 10, a patient with leukemia in family 13 and patients with multiple primary cancers in families 13 and 28. However, these families did not meet the criteria for LFS (23). Colorectal cancer and uterine endometrial cancer are the component tumors in HNPCC (11). There were colorectal cancer patients in families 9 and 22, whereas there were uterine cancer patients in families 8, 12 and 16. Thus, it is possible that these families were affected by HNPCC. However, none of these families met the Amsterdam criteria for HNPCC (22).

There were 11 families in which more than two family members were affected by gastric cancer under 50 years of age, suggesting the presence of common factors for the risk of early onset gastric cancer in those families. In particular, there were five or six gastric cancer patients in families 3, 5, 8 and 15. In addition, there were gastric cancer patients only in families 5 and 15.

In 29 cases, paraffin-embedded tumor samples of the probands were available for pathological examination. Gastric tumors were pathologically examined by two or more pathologists independently and standardized by one of the authors (H.S.) based on the General Rules of the Japanese Research Society for Gastric Cancer and the Lauren classification (24,25). According to Lauren's criteria, 17 cases (59%) were of the intestinal type and 12 cases (41%) were of the diffuse type. Compared with the current distribution of the histological subtypes of gastric cancer (26), a slight dominance of the intestinal type was apparently noted.

DNA was extracted from normal mucosae and tumorous portions dissected from paraffin-embedded surgical specimens as reported previously (27). In 13 of 31 cases (probands of families 3–8, 11, 13, 14, 16, 17, 27 and 29), DNA samples were available for genetic analysis. The RER phenotype was examined at six microsatellite loci, D1S191 (1q), D2S136 (2p), D3S1067 (3p), D5S421 (5q), D9S162 (9p) and TP53 (17p) (15,28,29). Three of the 13 cases (23%) showed RER at three or more of the microsatellite loci examined (Figure 2 and Table III). Histologically, two of the three cases with the RER phenotype were of the intestinal type and the other was of the diffuse type. The two intestinal types were in the early stage and the diffuse type was in the advanced stage.

Exons 2–11 of the *p53* gene and exons 3–16 of the *E-cadherin* gene were amplified for single-strand conformation polymorphism (SSCP) analysis using DNA extracted from normal mucosae. Primer sequences are available upon request (30–32). The procedures of PCR–SSCP analysis were described previously (12,13,15), except that PCR products of *E-cadherin* were electrophoresed on a polyacrylamide gel in 0.5× TPE (30 mM Tris, 20 mM PIPES and 1 mM Na_2EDTA, pH 6.8) (33). DNAs corresponding to the shifted bands were directly sequenced with the *fmol* DNA cycle sequencing system (Promega). No germline *p53* mutations were detected, however, one conservative missense mutation (Figure 3) and one polymorphism of the *E-cadherin* gene were identified in these 13 cases. In case 8, one of the three diffuse types, a G→T nucleotide change was detected at position 185 (Table III). This change was associated with an exchange of an amino acid (Gly→Val) at codon 62 in exon 3, located in the precursor sequence (34,35). A silent T/C polymorphism at codon 692 in exon 13 was found at an allele frequency of 0.69/0.31.

Here we have recruited and characterized various familial gastric cancer cases from the analysis of family histories among 3632 patients with gastric cancer. Familial cases were selected

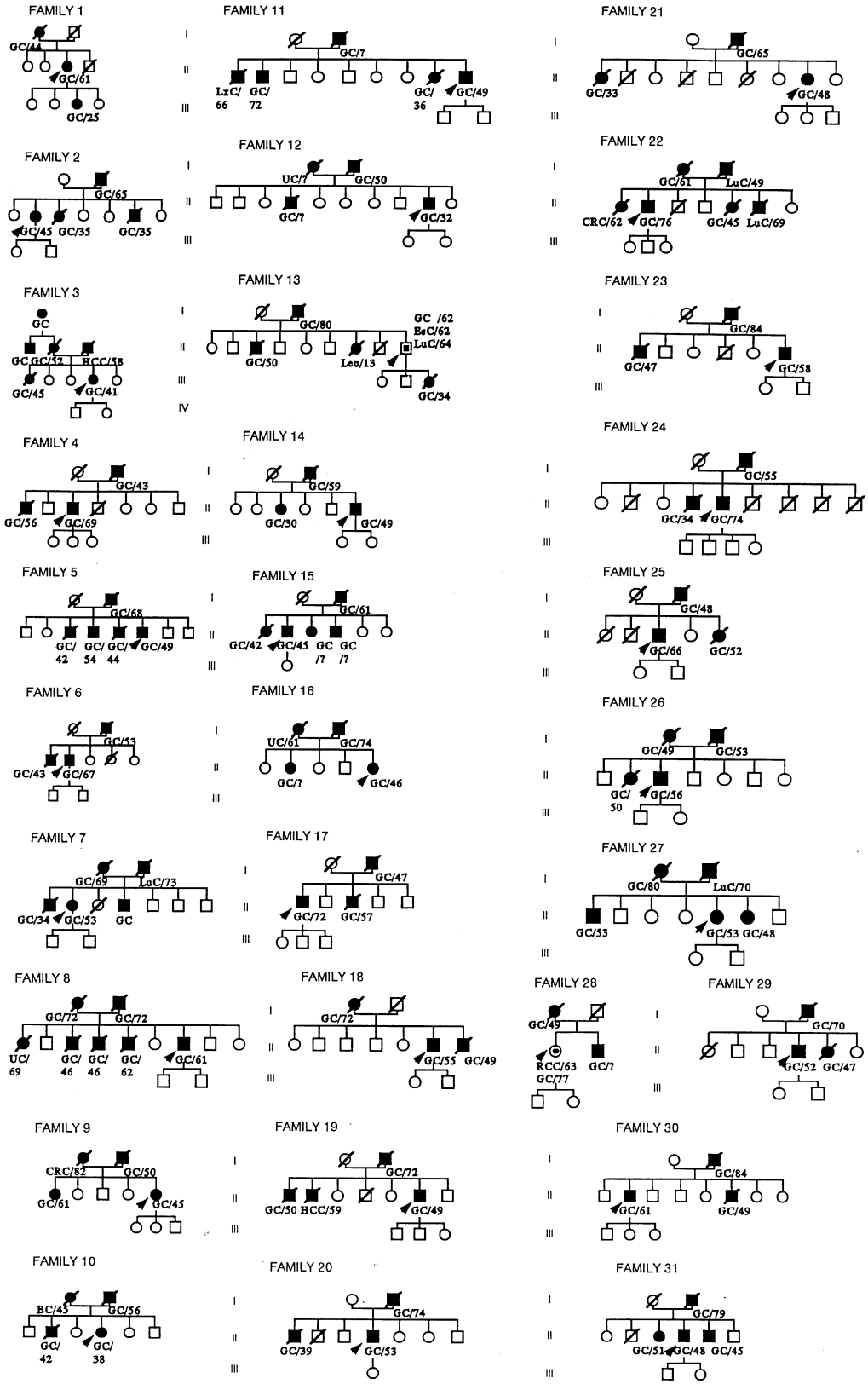

Fig. 1. Pedigrees of 31 families meeting the criteria for familial aggregation of gastric cancer. ○, ●, female; □, ■, male; symbols with slashes indicate deceased individuals. Open symbols indicate no neoplasm and filled symbols represent persons with cancers. GC, gastric cancer; LuC, lung cancer; UC, uterine cancer; CRC, colorectal cancer; HCC, hepatocellular carcinoma; BC, breast cancer; EsC, esophageal cancer; RCC, renal cell carcinoma; LxC, laryngeal cancer; Leu, leukemia. Numbers after the symbols for the type of cancer indicate age at death (family members) or age at diagnosis (probands). Arrowheads indicate probands.

based on the criteria resembling the Amsterdam criteria for HNPCC. The results indicated that ~1% of gastric cancer patients have histories of familial aggregation of gastric cancer. There were no families that fitted the clinical criteria for HNPCC and LFS. Instead, gastric cancer is predominantly clustered in these families. As with the histological types of gastric cancer in familial cases, a slight dominance of the intestinal type was observed. These results indicate the presence of unique families in which gastric cancer is predominantly clustered in family members.

Genetic factors for susceptibility to gastric cancer are presently unknown except for germline *E-cadherin* mutations (20,21). However, several lines of evidence indicate that gastric cancer frequently occurs in patients with HNPCC and LFS (11–14). The RER phenotype was detected in three of the 13 cases examined. Although there were no colorectal cancers in these three families, the RER phenotype was detected in two cases of early stage intestinal-type tumors. Early acquisition of the RER phenotype was also observed in HNPCC and other familial gastric cancer cases (15,36,37). Furthermore, the RER phenotype detected in this study corresponds to severe RER which is seen in HNPCC tumors (36) and the intestinal type histology is a characteristic of gastric cancers in HNPCC (38). Thus, it is possible that familial aggregation of gastric cancers in these cases is caused by inherited mismatch repair deficiency. However, the incidence of the RER phenotype detected in this series nearly coincides with those in our previous series of familial and sporadic gastric cancer cases (15,37). Thus, most familial gastric cancer cases would be genetically distinct from HNPCC. We previously identified two LFS families in which several family members were affected by gastric cancers at young ages (12,13), suggesting that germline *p53* mutations are present in families with aggregation of gastric cancer. However, the present results indicate that *p53* mutations are rare in familial gastric cancer.

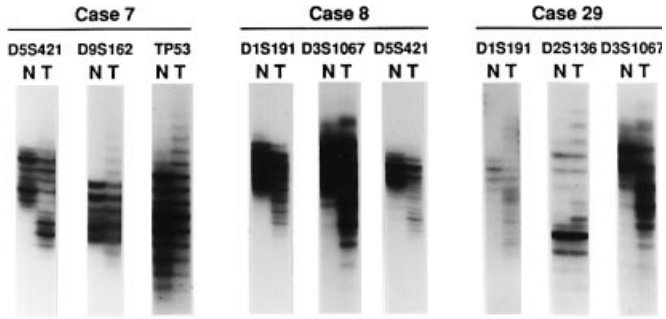

Fig. 2. RERs detected in three familial gastric cancer cases. Genomic DNA was extracted from gastric cancer (T) and corresponding normal gastric mucosa (N) of cases 7, 8 and 29. Microsatellite loci examined are shown on top.

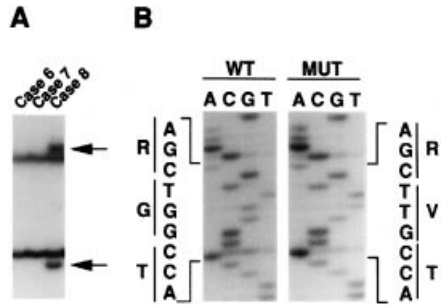

Fig. 3. Germline *E-cadherin* mutation detected in a familial gastric cancer case. (**A**) PCR–SSCP analysis covering the forward side of exon 3 for cases 6–8. The mutant allele in case 8 is indicated by arrows. (**B**) Nucleotide sequence analysis of wild-type (WT) and mutant (MUT) DNA fragments. The substitution of glycine (GGT) for valine (GTT) at codon 62 in exon 3 was detected in the mutant allele.

A missense mutation (Gly→Val) in the precursor sequence of the *E-cadherin* gene was detected in a diffuse-type familial case. This nucleotide change was not detected in other cases and has not been previously reported (20,39–41). Since Gly and Val belong to the same amino acid group and the precursor sequence is cleaved off by processing before delivery to the cell surface (34,35,42), the effect of this mutation on the function of E-cadherin protein is unclear. Thus, it is possible that the genotype of Val62 is a rare genetic polymorphism and not a germline mutation. In the present study, only three diffuse-type cases were available for genetic analysis and histological types of gastric cancers in family members of three probands with the diffuse type were unclear. Thus, further analysis of familial diffuse-type gastric cancers will be necessary to elucidate the pathogenetic significance of germline *E-cadherin* mutations in familial gastric cancer.

In conclusion, familial aggregation of gastric cancer occurs in ~1% of gastric cancer patients. Although the genetic factors resulting in this aggregation have been unclear, the present study indicates that germline mutations of the DNA mismatch repair, *p53 and E-cadherin* genes do not significantly contribute to such a clustering. Thus, genetic linkage analysis might facilitate the identification of other genetic factors responsible for susceptibility to gastric cancer. However, we should also consider here several other possibilities for such an aggregation. Familial

Table III. RER phenotype and germline *E-cadherin* mutation in familial gastric cancer cases

Case[a]	Age/sex	Depth	Histology[b]	RER phenotype		Germline *E-cadherin* mutation				
				Locus of RER	Rate[c]	Nucleotide position[d]	Exon	Codon	Nucleotide change	Amino acid change
7	53/F	Early	Intestinal	D1S191, D2S136, D3S1067, D5S421, D9S162, TP53	6/6					
8	61/M	Advanced	Diffuse	D1S191, D3S1067, D5S421, D9S162, TP53	5/6	185	3	62	GGT→GTT	Gly→Val
29	52/M	Early	Intestinal	D1S191, D2S136, D3S1067, TP53	4/5					

[a]Case number indicates the proband of the family with the same number.
[b]According to Lauren's criteria.
[c]No. loci showing RER/no. loci examined.
[d]Numbering is started from the A in the start codon of the cDNA sequence.

aggregation could occur by chance alone due to the high incidence of gastric cancer in Japan. Alternatively, it is also possible that there are environmental factors to reinforce the aggregation of gastric cancer in families.

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