RAPID COMMUNICATION Jpn. J. Cancer Res. **91**, 875–879, September 2000

Absence of Germline *CHK2* Mutations in Familial Gastric Cancer

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Recently, the *CHK2* gene was identified as being a candidate gene responsible for Li-Fraumeni syndrome (LFS). Gastric cancer is often clustered in families with LFS, so it is possible that germline *CHK2* mutation is also present in familial gastric cancer (FGC). We therefore defined the genomic structure of the *CHK2* gene, designed intronic primers, and searched for germline *CHK2* mutations in 25 FGC cases by polymerase chain reaction-single strand conformational polymorphism analysis of the entire coding region. In all of the 25 cases, at least two siblings had histories of gastric cancer. There were no FGC cases that showed germline *CHK2* mutations. Thus, it was indicated that germline *CHK2* mutations do not contribute to the familial clustering of gastric cancer.

Key words: CHK2 — Germline mutation — Familial gastric cancer

Gastric cancer remains a major cause of cancer death worldwide. Epidemiologically, there is familial aggregation of gastric cancers, as with colon, breast and several other cancers.[1–8] Inherited genetic alterations as well as environmental factors may be involved in the occurrence of such aggregations, but information has been limited to date.[9–13] Recently, germline mutations of the *E-cadherin* gene and the mismatch repair gene were identified in some familial gastric cancer (FGC) kindreds.[10–12] However, since such mutations have been detected only in a small subset of FGCs, we should consider other genetic factors in relation to the familial aggregation of gastric cancers.[13–16] We and others previously reported that gastric cancers are often clustered in families with Li-Fraumeni syndrome (LFS).[17–21] Thus, germline mutations of the genes responsible for LFS could also be a genetic factor for familial aggregation of gastric cancer. Germline *p53* mutation is a causative genetic event for LFS, but accounts for only about 60–70% of LFS.[22–24] Therefore, the presence of other genes responsible for LFS has been considered. Recently, germline mutations of the *CHK2* gene were found in three LFS families without germline *p53* mutations.[25] The *CHK2* gene was identified as a human homologue of the *Saccharomyces cerevisiae Rad53* and *Schizosaccharomyces pombe Cds1* checkpoint genes.[26] Human CHK2 kinase acts downstream of ATM and stabilizes p53 and Cdc25C proteins, which are key players in the cell cycle checkpoint.[27–32] Thus, a considerable fraction of LFS with the wild-type *p53* gene might be caused by germline *CHK2* gene mutations. Germline *CHK2* mutation may also be one of the genetic factors responsible for FGC. Thus, we examined 25 cases of FGCs for germline *CHK2* mutations by polymerase chain reaction-single strand conformational polymorphism (PCR-SSCP) analysis.

Since the genomic structure of the *CHK2* gene has not been determined, we first determined it by using the information on DDBJ/Genbank/EMBL DNA databases (accession No. AL117330 and AL121825). The *CHK2* gene consisted of 14 coding exons. Thus, intron-based primers for 14 exons covering the entire coding region were designed (Fig. 1). We then searched for germline *CHK2* mutations by PCR-SSCP analysis using genomic DNAs extracted from peripheral blood samples of 25 FGC cases.

Peripheral blood samples were obtained with informed consent from patients with FGC at the National Cancer Center Hospital, Tokyo, in 1998 and 1999. Family histories of patients were obtained from the patients and/or their family members at the time of hospitalization. In all 25 cases, at least one sibling of the patients had a history of gastric cancer. The number of gastric cancer patients among first- and second-degree relatives of the probands ranged from two to six. Twelve families (48%) also met the following criteria, which we previously defined[17]: (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 (Table I). Since we defined these criteria in a way analogous to that in the case of hereditary non-polyposis colorectal cancer (HNPCC), families con-

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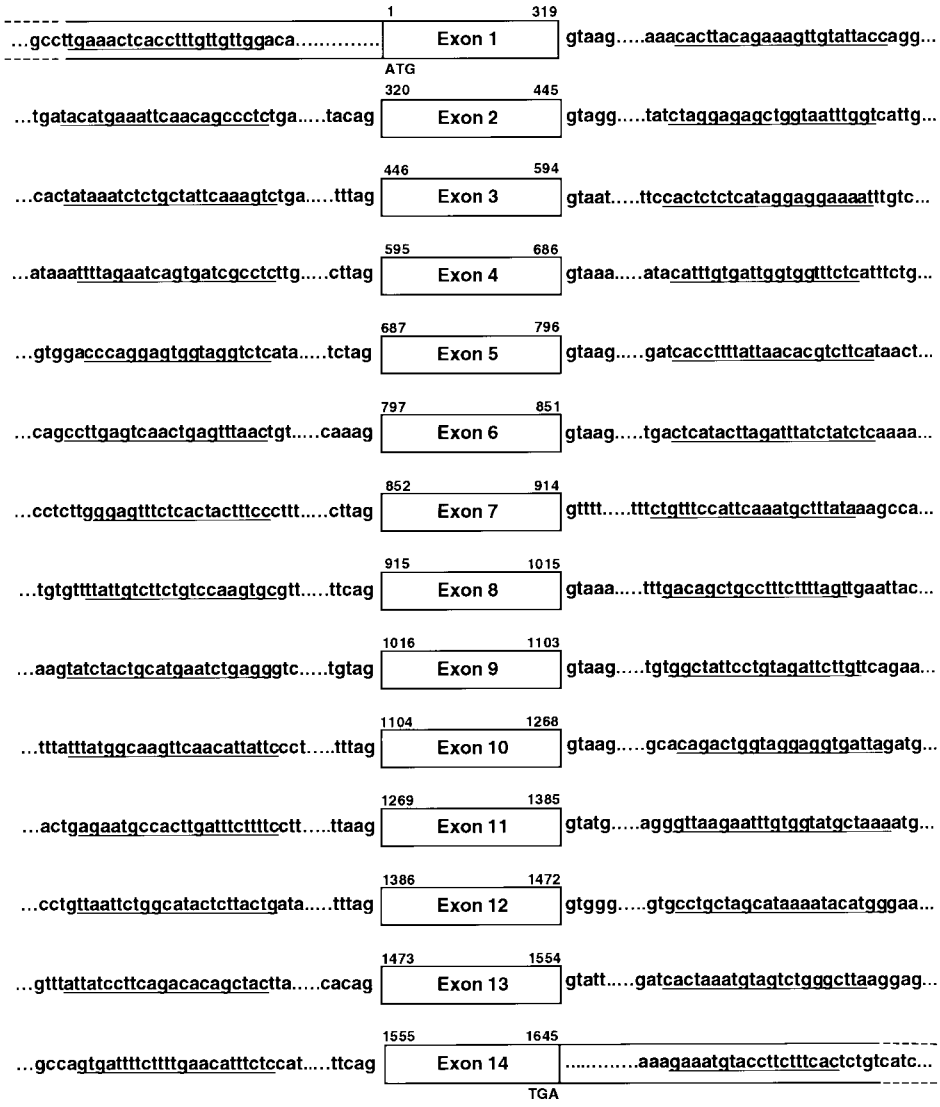

Fig. 1. Genomic structure of the *CHK2* gene and oligonucleotide primer pairs used for *CHK2* gene amplification. Primer sequences are underlined. The exon containing the ATG start codon was considered as exon 1.

forming to these criteria were likely to have genetic backgrounds favoring high susceptibility to gastric cancer in an autosomally dominant fashion.

Genomic DNA was extracted from the peripheral blood samples by proteinase K digestion and phenol-chloroform extraction. DNA samples from healthy volunteers were also used as controls. PCR-SSCP analysis was performed as described in the legend to Fig. 2. No band shifts were observed in any exon examined in any of the FGC samples analyzed, although a heterozygous silent A/G polymorphism was detected at codon 84 in exon 1 in control samples, as previously reported[25] (Fig. 2). Thus, we concluded that germline *CHK2* mutation is rare or not present in FGC.

The present result indicates that germline *CHK2* mutations do not contribute to familial clustering of gastric cancer. Thus, we should further search for other genetic factors responsible for familial aggregation of gastric cancer. Gastric cancer can be divided into two major histological types, intestinal type and diffuse type. Germline *E-cadherin* mutations have been identified to date only in a small subset of families with aggregation of diffuse type gastric cancer, and not at all in families with aggregation of intestinal type gastric cancer.[7, 10, 11, 17] The results indi-

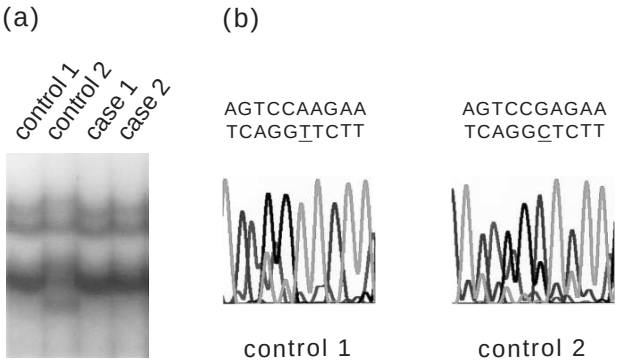

Fig. 2. A *CHK2* polymorphism detected in control materials. (a) PCR-SSCP analysis for exon 1 of the *CHK2* gene. A hundred nanograms of genomic DNA was suspended in a total volume of 20 μl of PCR buffer, containing 10 m*M* Tris-HCl (pH 9.0), 50 m*M* KCl, 1.5 m*M* $MgCl_2$, 200 n*M* each primer, 200 μM deoxynucleotide triphosphate, 2.5 μCi of [α-^{32}P]dCTP (Amersham Pharmacia Biotech, Foster City, CA), and 0.75 unit of Taq DNA polymerase (Amersham Pharmacia Biotech). PCR conditions were 60 s at 95°C, 60 s at 56°C, and 90 s at 72°C, for 35 cycles, followed by 10 min at 72°C. PCR products were diluted 10-fold with formamide dye solution, denatured at 95°C for 10 min, loaded on non-denaturing polyacrylamide gel containing 5% polyacrylamide (99:1 acrylamide to bisacrylamide) and Tris/PIPES/EDTA (TPE) (30 mmol/liter Tris, 20 mmol/liter piperazine-N,N-bis-[2-ethanesulfonic acid], and 1 mmol/liter Na_2EDTA; pH 6.8), electrophoresed in TPE buffer at 15°C, and exposed to Kodak XAR films for 24 to 48 h at −80°C. (b) Direct sequencing of the shifted band. PCR products showing different mobilities were purified using a QIA quick-spin PCR purification kit (QIAGEN, Tokyo) and directly sequenced in both directions with BigDye terminator cycle sequencing pre-mix kits (Amersham Pharmacia Biotech) and the ABI 310 DNA Sequence System (Perkin-Elmer, Tokyo). The sites of the genetic polymorphism are underlined.

Table I. Characteristics of Families with Aggregation of Gastric Cancer

No.	Case examined		Number of gastric cancer patients	Sibpair	Meeting the criteria we defined[17]
	Age of onset	Sex			
1	61	male	5	yes	yes
2	63	female	3	yes	
3	46	male	5	yes	yes
4	67	male	4	yes	yes
5	48	male	3	yes	yes
6	53	female	4	yes	yes
7	48	female	2	yes	
8	55	male	3	yes	yes
9	64	female	3	yes	yes
10	57	female	3	yes	yes
11	62	male	5	yes	yes
12	60	female	2	yes	
13	76	female	3	yes	
14	60	male	4	yes	
15	67	female	3	yes	yes
16	54	male	2	yes	
17	71	male	4	yes	
18	52	male	4	yes	
19	62	female	3	yes	yes
20	49	female	3	yes	
21	60	male	4	yes	yes
22	56	male	4	yes	
23	55	male	2	yes	
24	50	female	6	yes	
25	58	male	3	yes	

cate that the responsible genes are different between diffuse type and intestinal type FGCs. Accordingly, the criteria for familial diffuse gastric cancer and those for familial intestinal gastric cancer were individually defined by the International Gastric Cancer Linkage Consortium (IGCLC).[7] In most cases in this study, histological data could be obtained only from index cases and not from other family members. Thus, it is unclear which of the 25 FGC cases meet the criteria for familial diffuse gastric cancer or those for familial intestinal gastric cancer.

Therefore, it would be very important to collect more detailed clinicopathological data of each family member with gastric cancer for the identification of FGC cases with common genetic backgrounds. We should also consider several environmental factors putatively causative of familial aggregation, as well as clustering by chance owing to the high incidence of gastric cancer in Japan.

This work was supported in part by a Grant-in-Aid from the Ministry of Health and Welfare of Japan. K. Kimura and K. Shimizu are recipients of research resident fellowships from the Foundation for Promotion of Cancer Research.

(Received June 26, 2000/Revised July 28, 2000/Accepted August 1, 2000)

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