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 genic 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. Inherited genetic alterations as well as environmental factors may be involved in the occurrence of such aggregations, but information has been limited to date. Recently, germline mutations of the E-cadherin gene and the mismatch repair gene were identified in some familial gastric cancer (FGC) kindreds. 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. We and others previously reported that gastric cancers are often clustered in families with Li-Fraumeni syndrome (LFS). 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. 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. The CHK2 gene was identified as a human homologue of the Schizosaccharomyces pombe Cds1 checkpoint genes. Human CHK2 kinase acts downstream of ATM and stabilizes p53 and Cdc25C proteins, which are key players in the cell cycle checkpoint. 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: (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-
forming to these criteria were likely to have genetic back-
grounds 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 sam-

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.

cluded that germline CHK2 mutation is rare or not present
in FGC.

The present result indicates that germline CHK2 muta-
tions do not contribute to familial clustering of gastric can-
cer. Thus, we should further search for other genetic
factors responsible for familial aggregation of gastric can-
cer. Gastric cancer can be divided into two major histolog-
ical 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. The results indi-
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). 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 by chance owing to the high incidence of gastric cancer in Japan.

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