A 67-year-old man underwent two endoscopic submucosal dissection procedures, one for gastric adenoma and one for early gastric cancer. The follow-up endoscopy showed metachronous recurrence at the anterior wall of the lower body, for which he underwent a subtotal gastrectomy. Four first- or second-degree relatives in his family have been diagnosed with gastric or colon cancers. The patient underwent counseling and genetic testing to identify single nucleotide polymorphisms and indel variants for 31 genes by next generation sequencing. Five missense mutations were identified, one each in \textit{ATM}, \textit{BRIP1}, and \textit{EPCAM} and two in \textit{BRCA2}. These genetic alterations may be candidates for genetic causes of this familial cluster of gastric cancer. This study identified genes that, for the first time, can be potentially associated with an increased risk of familial gastric cancer among the Korean population. These results may be helpful in evaluating other genetic factors related to the etiology of gastric cancer.

\textbf{Key Words:} Genes; Mutation; Stomach neoplasms

\section*{INTRODUCTION}

Gastric cancer is the third most lethal cancer worldwide\textsuperscript{1} and one of the commonest cancers in South Korea. There are a range of risk factors for gastric cancer. They include environmental factors, such as an infection with \textit{Helicobacter pylori}, genetic factors, and behavioral factors, such as smoking, a high salt and nitrate intake.\textsuperscript{2} Although most gastric cancer shows sporadic mutation, about 10\% of the cases show familial aggregation.\textsuperscript{3} However, little is known about the genetic alterations to hereditary predisposition in those familial cases.

Despite high prevalence of gastric cancer among Korean populations, there are only small numbers of reported cases revealing genetic factors associated with familial gastric cancer. There is a recent study that has identified a \textit{CDH1} mutation in a patient diagnosed with signet ring gastric cancer.\textsuperscript{4} In another study, increased risk of gastric cancer was also demonstrated among patients with hereditary nonpolyposis colorectal cancer.\textsuperscript{5} Such limited number of reported cases may be the result of environmental and lifestyle risk factors that may possibly play more crucial roles in the development of gastric cancer than the genetic factors among Korean populations or lack of attention from clinicians to identify relevant genetic factors.

We herein report a case in which genetic mutations were identified on a patient who had a recurrent gastric cancer on a background of significant family history of gastric and colorectal cancer. The mutations identified were \textit{BRCA2}, \textit{ATM}, \textit{BRIP1} and \textit{EPCAM} and we reviewed
the literature on each germinal mutation on line with the clinical significance they have.

**CASE REPORT**

A 67-year-old male visited to our primary care clinic for upper esophagogastroduodenoscopic examination as a routine follow-up of diagnosed gastric adenoma and early gastric cancer in December 2016. This is on a background of the two episodes of endoscopic submucosal dissections. He underwent endoscopic submucosal dissection for excision of a slightly depressed lesion at the lesser curvature of the lower body of the stomach, which revealed well differentiated adenocarcinoma which confined to the mucosa without lymphovascular invasion in March 2015 and another endoscopic submucosal dissection of gastric adenoma at the greater curvature of the lower body of the stomach was followed in June 2015 at St. Paul’s Hospital in South Korea (Fig. 1). His medical history includes diabetes for which he has taken glimepiride for 5 years. He smokes 40 pack years and drinks 4 – 5 standard drinks per week. He underwent colonoscopic polypectomy of several adenoma in December 2014 at our clinic. At a follow-up endoscopy, there was a metachronous lesion at the greater curvature of the lower body of the stomach (Fig. 2). Biopsies confirmed signet ring cell adenocarcinoma, and for this reason, a subtotal gastrectomy was performed at St. Paul’s Hospital. The histopathological examination revealed poorly differentiated adenocarcinoma with no lymph node metastasis (pT1N0M0) (Fig. 3).

His family pedigree (Fig. 4) revealed a strong history of gastric and colorectal cancers. His father was diagnosed with colorectal cancer at the age of 70, whereas his mother was diagnosed with gastric cancer at the age of 49. He has one first-degree relative with colorectal cancer.

**Fig. 1.** Endoscopic findings. (A) An early gastric cancer lesion at the lesser curvature of the lower body of the stomach. (B) Another gastric adenoma lesion at the greater curvature of the proximal antrum of the stomach.

**Fig. 2.** Endoscopic findings. (A) An 8 mm sized round elevated metachronous lesion is detected at the lesser curvature of the lower body of the stomach. (B) Hemoclips are applied for locating the lesion.
who was diagnosed at the age of 57 and one with a gastric polyp. One of his second-degree relative was diagnosed with gastric cancer at the age of 60. He has 44 and 42 year old sons who have not been diagnosed with any malignancy. Considering his history of gastric cancer recurrence and a strong family history of gastric and colon cancer, a genetic testing on his blood sample was performed. Following verbal and written informed consent, genomic DNA was extracted from a peripheral blood sample 1 microgram of fragmented DNA was prepared to construct libraries with the SureSelect Focused exome Kit (Agilent, Inc., USA) using manufacturer’s protocol. After this, the qualified genomic DNA sample was randomly fragmented by Covaris followed by adapter ligation, purification, hybridization and PCR. Captured libraries were subjected to Agilent 2100 Bioanalyzer to estimate the quality, and were loaded on to the Illumina HiSeq2500 (TheragenEtex Bio Institute, Suwon, Korea) according to the manufacturer’s recommendations. Raw image files were processed by HCS ver. 1.4.8 for base-calling with default parameters and the sequences of each individual were generated as 101 bp paired-end reads. Sequence reads were aligned to the human reference genome build 37, using Novoalign (novocraft.com). The quality of the reads was checked using fastQC (ver. 0.10.1) and the ones with low-quality bases below Q20 were removed using Cutadapt (ver. 1.8.1). High quality reads were then aligned to the human reference genome hg19 using Burrows Wheeler Aligner (BWA) (ver. 0.7.12). During the process, duplicated reads were further removed using PicardTools (ver. 1.98). For the unique reads, the SNP and indel variants for 31 genes (APC, ATM, BARD1, BMPRIA, BRCA1, BRCA2, BRIPI, CDH1, CDK4, CDKN2A, CHEK2, EFCAM, MLH1, MRE11A, MSH2, MSH6, MUTYH, NBN, PALB2, PMS2, PSS1, PTEN, RAD50, RAD51C, RAD51D, RET, SMAD4, STK11, TP53,
Table 1. Five Germline Variants Found in a Patient with Familial Gastrointestinal Cancer

| ID      | Gene CDS change | AA change     | Type     |
|---------|-----------------|---------------|----------|
| rs659243 | ATM c.5948A>G   | p.Asn1983Ser  | Missense |
| rs144848 | BRCA2 c.1114A>C | p.Asn372His   | Missense |
| rs169547 | BRCA2 c.7537T>C | p.Val2466Ala  | Missense |
| rs4986764| BRIP1 c.2755T>C | p.Ser919Pro   | Missense |
| rs1126497| EPCAM c.344T>C  | p.Met115Thr   | Missense |

CDS, coding DNA sequence; AA, amino acid; ATM, ataxia telangiectasia mutated gene; BRCA, breast cancer gene; BRIP, BRCA1-interacting protein; EPCAM, epithelial cell adhesion molecule gene.

VHL) were identified using GenomeAnalysisTK (ver. 2.3.9). The detailed annotation for the variants were performed using SnpEff (ver. 4.1). Allele frequency for the variants was analyzed based on 1000 Genome Project (phase 3), EXAC and ESP6500. Hereditary cancer mutations were selected by the Clinvar database, and the somatic mutations, which are frequently identified in Korean population, were selected. Finally, we selected a total of 5 mutations and verified the mutation with Sanger sequencing. Selected Single Nucleotide Variants (SNVs) were validated by Sanger sequencing. Each PCR product was cycle-sequenced using BigDye® Terminator ver. 3.1 (Applied Biosystems, Foster City, CA, USA), and the samples were then sequenced on the automated capillary sequencer 3130X1 Genetic Analyser (Applied Biosystems).

Upon the genetic testing, BRCA2, ATM (ataxia-telangiectasia mutated) gene, BRIP1 mutation and EPCAM mutation were identified (Table 1). All variants were missense mutations. We have currently recommended a genomic test for his two sons in their 40 years.

**DISCUSSION**

Due to its high prevalence of gastric cancer in South Korea,

even though there is a strong family history of gastric cancer in certain patients, clinicians make less suspicion on those patients having a genetic predisposition. For this reason, it is uncommon to perform genetic testing on patients with the positive family history of gastric cancer in Korea. The patient in our case, on the other hand, received a genetic testing due to the occurrence of metachronous gastric cancer recurrence and the strong family history. The genetic testing performed in our case showed 5 missense mutations.

The BRCA2 is a tumor suppressor protein located on the long (q) arm of chromosome 13 at position 12.3. It is involved in DNA damage recognition, double-strand break repair, checkpoint control, transcription regulation and chromatin remodeling, and its dysfunction from deleterious mutations results in instability of cellular genetic materials. Not only do BRCA1/2 mutation involve in breast and ovarian cancer, but they also increase risk of developing prostate, pancreas, stomach, colon, peritoneal cancers, and furthermore, hematologic malignancies, such as leukemia and lymphoma. For gastric cancer, the RR is 1.69 (95% confidence interval, 1.21 ∼ 2.38) in BRCA1/2 mutation carriers according to the meta-analysis of more than 30 studies. However, a clear mechanism of gastric carcinogenesis of BRCA2 germ-line mutation has not been fully explained. In our case, p.A372H (c.1114A>C) and p.V2466A (c.7397T>C) were identified in BRCA1 gene on the patient.

ATM gene is also a tumor suppressor gene which is related to ataxia-telangiectasia as its name suggests. Significant association between the ATM and gastric cancer has not been established yet. However, Huang et al.12 identified several ATM mutations in a Chinese family with a history of gastric cancer and also suggested that ATM mutation may predispose to familiar gastric cancer. Furthermore, Helgason et al.13 found a loss of function mutations in ATM on Europeans with gastric cancer. In our patient, a missense mutation, p.A1983S (c.5948A>G) was identified in ATM gene, and it is a novel mutation which has not been reported elsewhere.

EPCAM is a cell surface glycoprotein of 40 kd which plays a role in proliferation, migration and a cell cycle regulation of cancer cells. EPCAM mutation is clinically important as they can cause hereditary nonpolyposis colorectal cancer as known as Lynch syndrome. Recently, one meta-analysis of reviewing the association between gastric cancer and EPCAM revealed EPCAM might be the worse prognostic factor as well as was overexpressed in gastric cancer patients than control group.

BRIP1 (also known as BACH1 or FANCJ) is a tumor suppressor gene, and its mutation can result in hereditary
breast cancer, ovarian cancer and Fanconi anemia. Fanconi anemia is a rare autosomal recessive disorder associated with progressive bone marrow failure, skeletal abnormality and cancer.16 There was no literature of review or case report about the relationship between BRIP1 and gastric cancer so far.

To find out whether these variants positively correlate with the carcinogenesis or familial aggregation of the cancer that patient and his family had, a genetic testing must be performed on the patient’s family members. However, it was practically difficult for many reasons. Some of the family members have already passed away. Moreover, some of them live interstate, so it was difficult to be in contact with them.

Several studies about metachronous recurrence after endoscopic submucosal dissection of early gastric cancer reported the median interval between the discovery of metachronous cancer and the initial endoscopic resection was 2 ~ 3 years, which was similar to the current case as 20 months. The risk factors of recurrence were H. pylori infection, male gender and microsatellite instability.17,18 Considering one report describing patients with a positive family history of cancer tended to have a greater risk of recurrence after subtotal gastrectomy of early gastric cancer,19 we might suggest the family history of our patient contribute to cancer recurrence.

In our case, the patient underwent subtotal gastrectomy and did not receive any chemotherapy or radiotherapy. If more studies are carried out to further investigate a gastric carcinogenesis of germinal mutations, a therapeutic approach such as target therapy specific to gastric cancer associated with several germinal mutations might be considered.

In summary, we describe a very unusual case of recurrent gastric cancer where genetic analysis was performed in Korea. This report suggests the possible correlation between some genetic mutations and recurrent gastric cancer. Even though more studies on the relationship between these mutations and gastric cancer are needed for proving clinical significance, this report could contribute to future genetic studies on gastric cancer, and this is particularly important in countries, such as South Korea, where the incidence and mortality rates of gastric cancer are high.

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