Introduction

The rate of occurrence of colorectal cancer in Korean females has been consistently rising in the latest decade. In 2010, colorectal cancer ranks as the third most common cancer (10.3%) in Korean females, with the newest patients being 10,170. Meanwhile, five-year survival rate has been ascending either; the relative ratio of five-year survival in Korean female in between 2006 and 2010 recorded 69.9% [1].

Hereditary nonpolyposis colorectal cancer (HNPCC), also known as Lynch syndrome, is inherited as autosomal dominant manner, and manifests in relatively younger age. It presents as a variety of malignancies such as endometrial cancer, colorectal cancer, and some other cancers that are known to be linked with microsatellite instability (MSI). This syndrome comes into existence by germline mutations on the DNA mismatch repair.
genes: MSH2, MLH1, MSH6, PMS1, and PMS2, among which MSH2, MLH1, and MSH6 mutations in the majority of cases [2].

According to Aarnio et al. [3], the endometrial cancer is the second most common presentation among HNPCC pedigree, following the colorectal cancer; while the occurrence rate of the endometrial cancer among general population is 1.3%, that of HNPCC pedigree reaches as high as 60%. In fact, endometrial cancer was included as diagnostic criteria of HNPCC, according to the Revised Bethesda guideline [4], and Amsterdam criteria II [5], justifying the necessity of the early screening more crucial.

Although the incidence of endometrial cancer is still low in Korea, it is the most common gynecologic malignancy in western countries. However, the incidence of endometrial cancer has increased rapidly in Korea [1]. Thus, endometrial cancer is a clinically important gynecological malignancy, and it is known to be genetically related to the HNPCC. However, the domestic data to prove its significance is still insufficient. For this reason, this study would like to highlight the genetic connection between double primary malignancies of colorectum and endometrium in patients who were registered in a single institution.

**Materials and methods**

1. Patients
There were 12 patients who were diagnosed of both colorectal and endometrial cancers in Seoul National University Hospital in between January 2004 and December 2013; a retrospective study was conducted for these patients. Data including MSI analysis, immunohistochemistry (IHC) staining, family history, and other accompanied diseases were collected by medical records. This study was conducted according to the Helsinki Declaration statement, and approved by the institutional review board (no. 1407-132-597).

2. Microsatellite instability analysis
For MSI analysis, polymerase chain reaction was conducted from DNA of the frozen tumor or normal tissue to verify the sequence of 2 mononucleotide repeats (BAT25 and BAT26) and 3 dinucleotide repeats (D2S123, D5S346, and D17S250) with specific primers for each kind (Bioneer, Daejeon, Korea). MSI was confirmed when the specimen showed either a longer or a shorter PCR product compared to the normal tissue of the patient. The specimen was sorted as microsatellite instability-high (MSI-H) when 2 or more of the 5 markers were found to have instability, whereas microsatellite stable were labeled when none of the markers contained instability. Other specimen that did not meet such criteria were classified as microsatellite instability-low, i.e., when only one marker contained instability [6].

3. Immunohistochemistry staining
IHC staining for MLH1 and MSH2 expression was performed to the slide from paraffin-embed tissue with the use of the antibodies against MLH1 and MSH2 as the primary antibodies, respectively: mouse monoclonal antibodies against hMLH1 (clone ES05, DAKO, Glostrup, Denmark) and MSH2 (clone FE11, Life Technologies, Seoul, Korea). HRP multimer-tagged secondary antibodies were then utilized. Specimens that lack either one of those markers were classified as the loss of mismatch repair (MMR) expression [7-9].

4. Mutation analysis
Mutation analysis was conducted on the volunteer candidates among those who showed both MSI-H and loss of MMR expression. Informed consent was obtained and the blood samples were collected from the patients in EDTA coated tubes. DNA was extracted and PCR was performed using primers specific for 19 coding exons of the MLH1 gene (reference sequence: NM_000249.3) and 16 coding exons of the MSH2 gene (reference sequence: NM_000251.2). The amplified products were sequenced on an ABI 3730 analyzer (Applied Biosystems, Foster City, CA, USA) using a BigDye Terminator v3.1 Cycle sequencing kit (Applied Biosystems) [10].

5. Statistical analysis
The frequencies and percentages of general characteristics by MSI analysis and IHC staining were calculated. All statistical test results were determined significant when two-sided significance levels of \( P \)-value was less than 0.05. Statistical analyses were performed using SPSS ver. 17 (SPSS Inc., Chicago, IL, USA).

**Results**
Characteristics of the patients were described in Table 1. For twelve patients who were diagnosed of both endometrial and colorectal cancers, the median age of detection in endometrial cancer was 52.5, and that in colorectal cancer was 54.5. Two of twelve patients were diagnosed of two kinds of malignan-
cies simultaneously, five were diagnosed of colorectal cancer precedent to endometrial cancer, and the rest five were diagnosed of endometrial cancer precedent to colorectal cancer. Pathology reports were missing in 4 cases, because the patients were referred to the Seoul National University Hospital for their secondary malignancies from other institutions; two patients presented unknown histopathology for endometrial cancer, and the other two were uncertain for the histopathology for their colorectal cancer. With these exceptions, among ten patients with a known pathology for the endometrial cancer, nine of them were confirmed as endometroid adenocarcinoma. In a similar manner, ten patients with a known pathology for the colorectal cancer were all confirmed as adenocarcinoma.

All twelve patients underwent MSI analysis, nine of whom were classified as MSI-H. Of these nine, eight were IHC-stained, and six of them were found to have the loss of MMR protein. Four out of these MMR protein losses were found in MSH2 locus (Fig. 1). None of the case revealed the loss of MMR protein in both MLH1 and MSH2 loci (Table 1).

Half of these 12 patients were diagnosed of either clinical HNPPC or HNPPC suspect; they were SNUOG 03, SNUOG 05, SNUOG 06, SNUOG 07, SNUOG 08, and SNUOG 12.

Table 1. Characteristics of patients with double primary cancers in endometrium and colorectum

| Patient no. | Endometrial cancer | Colon cancer | Other cancer | MSI analysis | IHC staining |
|-------------|-------------------|--------------|--------------|-------------|-------------|
|             | Diagnosed age     | Histology    | Diagnosed age | Histology   | Cancer      | Analysis   | Staining |
| SNUOG 01    | 54                | Endometrioid adenocarcinoma | 54 | Adenocarcinoma | None | MSI-H | Normal |
| SNUOG 02    | 51                | Endometrioid adenocarcinoma | 59 | Adenocarcinoma | None | MSI-H | MSH2 (+) |
| SNUOG 03    | 57                | Unknown      | 51 | Adenocarcinoma | Bladder cancer | MSI-H | Normal |
| SNUOG 04    | 64                | Unknown      | 77 | Adenocarcinoma | Gastric cancer | MSS | Not done |
| SNUOG 05    | 44                | Endometrioid adenocarcinoma | 45 | Adenocarcinoma | None | MSI-H | MSH2 (+) |
| SNUOG 06    | 47                | Endometrioid adenocarcinoma | 47 | Adenocarcinoma | Cervical cancer | MSI-H | MSH2 (+) |
| SNUOG 07    | 57                | Endometrioid adenocarcinoma | 55 | Adenocarcinoma | None | MSI-H | MLH1 (+) |
| SNUOG 08    | 46                | Endometrioid adenocarcinoma | 29 | Unknown | None | MSI-H | MLH1 (+) |
| SNUOG 09    | 70                | Clear cell adenocarcinoma | 63 | Adenocarcinoma | None | MSS | Not done |
| SNUOG 10    | 51                | Endometrioid adenocarcinoma | 65 | Adenocarcinoma | None | MSS | Not done |
| SNUOG 11    | 45                | Endometrioid adenocarcinoma | 55 | Adenocarcinoma | None | MSI-H | Not done |
| SNUOG 12    | 61                | Endometrioid adenocarcinoma | 48 | Unknown | Gastric cancer | MSI-H | MSH2 (+) |

MSI, microsatellite instability; IHC staining, immunohistochemical staining; MSI-H, microsatellite instability-high; MSS, microsatellite stable.

Fig. 1. Mismatch repair protein expression in MSH2 locus. (A) Retained MSH2 expression (×40). (B) Loss of MSH2 expression (×40).
Three patients (SNUOG 06, SNUOG 07, and SNUOG 08) were analyzed for a germline mutation, and all of them turned out positive for a known mutation. Genetic characteristics including medical and family history, were shown in Table 2. Two of them meet the Amsterdam criteria II, thereby could be clinically diagnosed as HNPCC [5], and the other one was a HNPCC suspect [11]. All of them showed both MSI-H and the loss of MMR protein.

### Discussion

In a sporadic cases of endometrial cancer, 2.6% of the patients lost their MMR protein expression; meanwhile, 8.3% of MSI-H group patients lost MMR expression [12]. Another study showed that 8.7% of patients with endometrial cancer were diagnosed of either HNPCC suspect or clinical HNPCC, and one-third of clinical HNPCC patients were found to have germline mutation [13]. The current study reveals that 50% of the patients with double malignancies are diagnosed of either HNPCC suspect or clinical HNPCC. Among study population, 75% of the patients with double primary cancers have MSI-H, and 75% of MSI-H patients lost MMR expression. This study evaluated genetic perspectives of the patients with double primary malignancies, and unveiled a stronger genetic aberration of them compared to the patients with endometrial cancer only.

When a germline mutation occurs within the DNA MMR gene locus, errors made during the replication of the genetic material cannot be corrected and therefore lead to MSI. When this defect happens in the genes concerning cell division or growth regulation, a malignant tumor could arise as a result [14]. The majority (38% to 78%) of the known germline mutation happens within a MSH2 gene locus in the endometrial cancer [15]. This study revealed that two of the three candidates were found to have MLH1 mutation. This result is thought to be a bias from the fact the analysis was done only for the volunteers who showed both MSI-H and loss of MMR protein. IHC staining is known to be a highly specific method for confirming MSH2 mutation [15]. Note that the rate of loss MSH2 is two thirds (67%) in this study, similar to the preceding studies.

In case of the patient SNUOG 06, family genetic counseling was conducted for two daughters and a younger sister of the patient; one of her daughter turned out positive for a MSH2 mutation. Screening using ultrasonography and colonoscopy is planned on the confirmed offspring.

There are some weak points in this study. The samples size was small, mainly due to both the financial manner of the genetic test and the preservation of tumor specimen. Furthermore, the candidates of test were not randomly selected and therefore it is difficult to generalize the results. When considering the facts that the incidence and prevalence of the endometrial cancer are low, and there has been no previous study, however, this study could play a role in setting up a hypothesis.

The current study is distinct in that it proved a genetic aberration in patients with both primary endometrial and colorectal cancers, by various methods including MSI analysis, IHC staining, and mutation analysis. Chances were higher to have a HNPCC when an individual has a double primary cancers in her endometrium and colorectum, compared to those whom with endometrial cancer only. It might be beneficial that a germline mutation analysis be done for endometrial cancer patients or their families, based on the family history of other type of cancers. Early detection and treatment became a more important matter of concern in oncology nowadays. This study supports the necessity of re-defining the high-risk groups in endometrial cancers as well as the early diagnosis in such patients. Furthermore, family members of the patient who were positive for germline mutation should be considered as targets of early screening.
Conflict of interest

No potential conflict of interest relevant to this article was reported.

Acknowledgments

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIP) (no. 2013R1A1A3012912).

References

1. National Cancer Information Center. Annual report of cancer statistics in Korea in 2011 [Internet]. Goyang: National Cancer Information Center; c2013 [cited 2015 Jan 27]. Available from: http://www.cancer.go.kr/ebook/84/PC/84.html.

2. Peltomaki P, Vasen H. Mutations associated with HNPCC predisposition: update of ICG-HNPCC/INSiGHT mutation database. Dis Markers 2004;20:269-76.

3. Aarnio M, Sankila R, Pukkala E, Salovaara R, Aaltonen LA, de la Chapelle A, et al. Cancer risk in mutation carriers of DNA-mismatch-repair genes. Int J Cancer 1999;81:214-8.

4. Rodriguez-Bigas MA, Boland CR, Hamilton SR, Henson DE, Jass JR, Khan PM, et al. A National Cancer Institute Workshop on Hereditary Nonpolyposis Colorectal Cancer Syndrome: meeting highlights and Bethesda guidelines. J Natl Cancer Inst 1997;89:1758-62.

5. Vasen HF, Watson P, Mecklin JP, Lynch HT. New clinical criteria for hereditary nonpolyposis colorectal cancer (HNPCC, Lynch syndrome) proposed by the International Collaborative group on HNPCC. Gastroenterology 1999;116:1453-6.

6. Loukola A, Eklin K, Laiho P, Salovaara R, Kristo P, Jarvinen H, et al. Microsatellite marker analysis in screening for hereditary nonpolyposis colorectal cancer (HNPCC). Cancer Res 2001;61:4545-9.

7. Shi SR, Key ME, Kalra KL. Antigen retrieval in formalin-fixed, paraffin-embedded tissues: an enhancement method for immunohistochemical staining based on microwave oven heating of tissue sections. J Histochem Cytochem 1991;39:741-8.

8. Hewitson TD, Wigg B, Becker GJ. Tissue preparation for histochemistry: fixation, embedding, and antigen retrieval for light microscopy. Methods Mol Biol 2010;611:3-18.

9. Shi SR, Liu C, Pootrakul L, Tang L, Young A, Chen R, et al. Evaluation of the value of frozen tissue section used as “gold standard” for immunohistochemistry. Am J Clin Pathol 2008;129:358-66.

10. Shin KH, Shin JH, Kim JH, Park JG. Mutational analysis of promoters of mismatch repair genes hMSH2 and hMLH1 in hereditary nonpolyposis colorectal cancer and early onset colorectal cancer patients: identification of three novel germ-line mutations in promoter of the hMSH2 gene. Cancer Res 2002;62:38-42.

11. Park JG, Vasen HF, Park YJ, Park KJ, Peltomaki P, de Leon MP, et al. Suspected HNPCC and Amsterdam criteria II: evaluation of mutation detection rate, an international collaborative study. Int J Colorectal Dis 2002;17:109-14.

12. Banno K, Susumu N, Yanokura M, Hirao T, Iwata T, Hirasawa A, et al. Association of HNPCC and endometrial cancers. Int J Clin Oncol 2004;9:262-9.

13. Lim MC, Seo SS, Kang S, Seong MW, Lee BY, Park SY. Hereditary non-polyposis colorectal cancer/Lynch syndrome in Korean patients with endometrial cancer. Jpn J Clin Oncol 2010;40:1121-7.

14. Boland CR, Koi M, Chang DK, Carethers JM. The biochemical basis of microsatellite instability and abnormal immunohistochemistry and clinical behavior in Lynch syndrome: from bench to bedside. Fam Cancer 2008;7:41-52.

15. Lu KH, Schorge JO, Rodabaugh KJ, Daniels MS, Sun CC, Soliman PT, et al. Prospective determination of prevalence of Lynch syndrome in young women with endometrial cancer. J Clin Oncol 2007;25:5158-64.