Tumor development in Japanese patients with Lynch syndrome

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Abstract

Background

Lynch syndrome (LS) patients have a high risk of developing various tumors. This study aimed to clarify the characteristics of tumors developing in LS patients.

Methods

This is a retrospective review of 55 LS patients treated at Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital.

Results

The median age at the diagnosis of the first malignant tumor and first LS-related tumor was 44 (range, 19–65) and 44 (range, 24–66) years, respectively. Of the 55 LS patients with developing malignant tumors, 45 (93.8%) developed an LS-related tumor as the first malignant tumor. Colorectal cancer (CRC) developed in 47 patients (85.4%), followed by endometrial cancer (n = 13, 56.5%) in females and gastric cancer (n = 10, 18.1%). In 6 gastric cancer patients, Helicobacter pylori was detected in resected specimens. Twenty-nine patients (52.7%) developed CRC and extra-colonic tumors; of these, 15 patients (48.3%) had mutations in MLH1, 10 (58.8%) in MSH2, and 4 (57.1%) in MSH6. At the age of 50, the cumulative incidence was 50.9% [95% confidence interval (CI), 36.9–63.3%] for CRC, 17.4% (95% CI, 5.2–35.6%) for endometrial cancer, and 5.5% (95% CI, 1.4–13.8%) for gastric cancer. Eight gastric cancer, one breast cancer patient, five bladder cancer patients, and one prostate cancer patient demonstrated loss of expression of the mismatch repair (MMR) protein; patients with thyroid cancer, spindle cell sarcoma, and giant cell tumors did not demonstrate this.
Conclusion
Gastric cancer incidence was high in Japanese patients with LS and associated with \textit{H. pylori} infection. MMR protein deficiency caused the development of malignant tumors in LS patients.

Introduction
Lynch syndrome (LS) is an autosomal dominant disorder caused by germline mutations in one of the mismatch repair (MMR) genes, including the \textit{MLH1} \cite{1}, \textit{MSH2} \cite{2}, and \textit{MSH6} genes \cite{3}. Inactivation of MMR genes by germline and somatic mutations leads to a high frequency of replication errors in microsatellite regions and repetitive sequences in the coding regions of various growth-related target genes \cite{4}, resulting in the development of various tumors. According to Amsterdam criteria II \cite{5}, LS-related tumors are colorectal, endometrial, small bowel, and ureter/renal pelvis cancers. In the Revised Bethesda Guidelines, the following tumors are also included as LS-related tumors: stomach, ovarian, pancreas, biliary tract, brain (usually glioblastoma) tumors, sebaceous gland adenomas, and keratoacanthomas \cite{6}.

Most recent data concerning tumor development in LS patients have been reported from Western countries \cite{7-13}; however, the details of tumors developing in LS patients from Asia have not yet been elucidated. Therefore, this study aimed to clarify the characteristics of tumors developing in Japanese patients with LS.

Methods
Patients
This study was approved by ethics committee of Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital. All patients have given written informed consents. Fifty-five LS patients were selected from 34 LS families. Patients in whom “pathogenic”/“likely pathogenic” germline mutations of MMR genes were not detected were excluded.

Clinical information, including sex, date of birth, occurrence of tumors, date at the diagnosis of tumors, and \textit{Helicobacter pylori} infection, was collected either from medical records or directly from patients.

According to the Revised Bethesda Guidelines, colorectal, endometrial, gastric, ovarian, pancreatic, ureter/renal pelvis, biliary tract, brain tumors, sebaceous gland adenomas, keratoacanthomas, and small bowel carcinomas were defined as LS-related tumors and other tumors were defined as non-LS-related tumors.

Immunohistochemistry
For immunohistochemical staining, the following primary antibodies were used: Purified Mouse Anti-\textit{MLH1} Monoclonal Antibody, clone G168-15 (BD Pharmingen, San Diego, CA) for \textit{MLH1}; Anti-\textit{MSH2} Antibody, clone FE11 (Calbiochem, La Jolla, CA) for \textit{MSH2}; Purified Mouse Anti-\textit{MSH6}, clone 44/\textit{MSH6} (BD Pharmingen) for \textit{MSH6}; and Purified Mouse Anti-\textit{PMS2}, clone A16-4 (BD Pharmingen) for \textit{PMS2}. Dilution rates of 50×, 50×, 100×, and 50×, respectively, were used. Staining was conducted using the DAKO EnVisionTM system (Agilent Technologies, Dako, Glostrup, Denmark), and diaminobenzidine (Sigma, St. Louis, MO) was used as the substrate chromogen. Normal colonic mucosa was used as the positive control.
**Statistical analysis**

Data are presented as total, median (range), mean (95% confidence interval), or percentage (95% confidence interval). Statistical analyses were performed using Fisher’s exact test and the Mann–Whitney U-test. Cumulative cancer risks were calculated using the Kaplan–Meier method, and to compare risks between the two groups, the log-rank test was used. \( P < 0.05 \) was considered statistically significant. All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan; http://www.jichi.ac.jp/saitama-sct/SaitamaHP.files/statmedEN.html), a graphical user interface for R version 3.4.1 (The R Foundation for Statistical Computing, Vienna, Austria) [14]. This interface is a modified version of R Commander version 2.4–0, which was designed to add statistical functions frequently used in biostatistics.

**Results**

Table 1 shows the incidence of developed tumors in the 55 LS patients. Causative MMR genes were as follows: \textit{MLH1} in 31 patients (56.3%), \textit{MSH2} in 17 (30.9%), and \textit{MSH6} in 7 (12.8%). Of the 55 these, 29 (52.7%) were females and 26 (47.3%) were males. The sex ratio was not significantly different across genes. In 45 (93.8%) of 48 LS patients with developing malignant tumors, LS-related tumors developed as the first malignant tumor.

Colorectal cancer was the most common malignant tumor \((n = 47, 85.4\%)\), followed by endometrial cancer \((n = 13, 56.5\%)\) and gastric cancer \((n = 10, 18.1\%)\). Of the 47 colorectal

| Table 1. Characteristics in LS patients. |
|------------------------------------------|
|                                          |
| All cases  | **MLH1** | **MSH2** | **MSH6** |
| Number of patients | 55 | 31 | 17 | 7 |
| Gender (Female: Male) | 29: 26 | 16: 15 | 8: 9 | 5: 2 |
| All tumor | 48 (87.3%) | | | |
| Colorectal | 47 (85.4%) | 26 (83.8%) | 17 (100%) | 4 (57.1%) |
| Endometrial ** | 13 (56.5%) | 6 (42.8%) | 3 (75%) | 4 (80%) |
| Gastric | 10 (18.1%) | 7 (22.5%) | 3 (17.6%) | 0 |
| Small bowel | 4 (7.2%) | 3 (9.6%) | 1 (5.8%) | 0 |
| Renal/urinary tract | 6 (10.9%) | 2 (6.4%) | 4 (23.5%) | 0 |
| Biliary | 2 (3.6%) | 2 (6.4%) | 0 | 0 |
| Pancreas | 1 (1.8%) | 1 (3.2%) | 0 | 0 |
| Ovary*** | 2 (11.7%) | 0 | 1 (20.0%) | 1 (50.0%) |
| Brain | 1 (1.8%) | 0 | 1 (5.8%) | 0 |
| Bladder* | 6 (10.9%) | 2 (6.4%) | 4 (23.5%) | 0 |
| Breast (only female)* | 4 (13.7%) | 3 (18.7%) | 1 (12.5%) | 0 |
| Cervix* | 1 (3.4%) | 1 (6.2%) | 0 | 0 |
| Thyroid* | 1 (1.8%) | 1 (3.2%) | 0 | 0 |
| Prostate* | 1 (3.8%) | 1 (6.6%) | 0 | 0 |
| Skin* | 1 (1.5%) | 0 | 1 (4%) | 0 |
| Lymphoma* | 1 (1.5%) | 0 | 1 (4%) | 0 |
| Sarcoma* | 1 (1.5%) | 1 (3.2%) | 0 | 0 |
| Bone tumor* | 1 (1.5%) | 1 (3.2%) | 0 | 0 |

*, non LS-related tumor
**, excluded patients who have had hysterectomy without malignancy
***, excluded patients who have had oophorectomy without malignancy

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cancer patients, 30 (63.8%) developed two or more colorectal tumors; the causative gene was \(MLH1\) in 15 patients, \(MSH2\) in 13 patients, and \(MSH6\) in 2 patients. Twenty-nine patients (52.7%) developed colorectal cancer and extra-colonic tumors; the causative gene was \(MLH1\) in 15 patients (48.3%), \(MSH2\) in 10 patients (58.8%), and \(MSH6\) in 4 patients (57.1%). Six patients had undergone hysterectomy without any gynecological malignancy. Excluding those 6 patients, 13 endometrial cancer patients (56.5%) were identified in total; the causative gene was \(MLH1\) in 6 patients (42.8%), \(MSH2\) in 3 patients (75%), and \(MSH6\) in 4 patients (80%). Breast cancer developed in 4 of the 29 females (13.7%). Of the 10 gastric cancer patients, all 6 treated in our hospital showed \(Helicobacter pylori\) in resected specimens.

Table 2 shows the first organ developing a malignant tumor for each gene. Colorectal cancer developed in 38% of the females and 85% of the males \((P = 0.0007)\). Endometrial cancer was the second most common in female patients (31%).

The median age at the diagnosis of malignant tumors is shown in Table 3. The median age at the diagnosis of the first malignant tumor and the first LS-related tumor was 44 (range: 19–66).

Table 2. The first organ developed malignant tumor.

| Tumor  | Male | Female |
|--------|------|--------|
|        | Colorectal | Gastric | Ureter | None | Colorectal | Endometrial | Ovary | Bladder | Bone* | Cervix | None |
| MLH1   | 15  | 0     | 0     | 0     | 6    | 4          | 0     | 0      | 1     | 1     | 4    |
| MSH2   | 7   | 1     | 1     | 0     | 4    | 2          | 1     | 1      | 0     | 0     | 0    |
| MSH6   | 0   | 0     | 0     | 0     | 2    | 1          | 3     | 0      | 0     | 0     | 1    |
| Total  | 22  | 1     | 1     | 2     | 11   | 9          | 1     | 1      | 1     | 1     | 5    |

*, non LS-related tumor

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Table 3. Median age at diagnosis of malignant tumors.

| Cancer type                  | Median age at diagnosis (range) |
|------------------------------|---------------------------------|
|                              | All, year | Female, year | Male, year |
| The first malignant tumor    | 44 (19–66) | 46 (19–63) | 43 (26–66) |
| The first LS-related tumor   | 44 (26–66) | 47 (29–63) | 43 (26–66) |
| Colorectal                   | 46 (26–74) | 53 (29–69) | 43 (26–74) |
| Endometrial                  | 50 (40–57) | 50 (40–57) | –          |
| Gastric                      | 62 (44–77) | 65 (60–77) | 47 (44–66) |
| Small intestine              | 62 (40–67) | 64 (60–67) | 51 (40–63) |
| Renal/urinary tract          | 58 (49–81) | 64 (56–81) | 51 (49–53) |
| Biliary                      | 63 (55–72) | 63 (55–72) | –          |
| Pancreas                     | 76          | 76          | –          |
| Ovary                        | 43 (36–50) | 43 (36–50) | –          |
| Brain                        | 49          | –           | 49         |
| Bladder*                     | 54 (50–82) | 59 (50–82) | 54 (53–55) |
| Breast*                      | 66 (44–78) | 66 (44–78) | –          |
| Cervix*                      | 68          | 68          | –          |
| Thyroid*                     | 65          | 65          | –          |
| Prostate*                    | 74          | –           | 74         |
| Skin*                        | 49          | –           | 49         |
| Lymphoma*                    | 89          | 89          | –          |
| Sarcoma*                     | 80          | 80          | –          |
| Bone cancer*                 | 19          | 19          | –          |

*, non LS-related tumor according to the Bethesda Guideline.

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Table 4. Age-specific cumulative incidence rates in Lynch syndrome patients.

| Cancer type       | Age, year | All, % | Female | Male | p-value | Female | Male | p-value | Female | Male | p-value |
|-------------------|-----------|--------|--------|------|---------|--------|------|---------|--------|------|---------|
| Colorectal        | 50        | 50.9   | 37.5   | 73.3 | 0.029   | 50.0   | 77.8 | 0.700   | 0.0    | 0.0  | 1.000   |
|                   | 60        | 65.5   | 50.0   | 86.7 | 0.0     | 100.0  | 88.9 | 0.0     | 60.0   | 0.0  | 0.0     |
|                   | 70        | 83.6   | 68.8   | 100.0| 0.0     | 100.0  | 100.0| 0.0     | 80.0   | 0.0  | 0.0     |
| Endometrial*      | 50        | 17.4   | 37.5   | –    | –       | 50.0   | –    | 20.0   | –      | –    | –       |
|                   | 60        | 56.5   | 50.0   | –    | –       | 50.0   | –    | 60.0   | –      | –    | –       |
|                   | 70        | 56.5   | 68.8   | –    | –       | 87.5   | –    | 80.0   | –      | –    | –       |
| Gastric           | 50        | 5.5    | 0.0    | 20.0 | 0.522   | 0.0    | 0.0  | 0.498   | 0.0    | 0.0  | –       |
|                   | 60        | 9.1    | 6.2    | 20.0 | 0.0     | 12.5   | 0.0  | 0.0     | 0.0    | 0.0  | 0.0     |
|                   | 70        | 14.5   | 12.5   | 20.0 | 0.0     | 25.0   | 11.1 | 0.0     | 0.0    | 0.0  | 0.0     |
| Small intestine   | 50        | 1.8    | 0.0    | 0.0  | 0.603   | 0.0    | 1.1  | 0.346   | 0.0    | 0.0  | –       |
|                   | 60        | 1.8    | 6.2    | 0.0  | 0.0     | 0.0    | 1.1  | 0.0     | 0.0    | 0.0  | 0.0     |
|                   | 70        | 7.3    | 12.5   | 6.7  | 0.0     | 0.0    | 1.1  | 0.0     | 0.0    | 0.0  | 0.0     |
| Renal/urinary tract| 50       | 1.8    | 0.0    | 0.0  | 0.945   | 0.0    | 1.1  | 0.542   | 0.0    | 0.0  | –       |
|                   | 60        | 5.5    | 6.2    | 6.7  | 0.0     | 0.0    | 1.1  | 0.0     | 0.0    | 0.0  | 0.0     |
|                   | 70        | 6.9    | 6.2    | 6.7  | 0.0     | 2.5    | 1.1  | 0.0     | 0.0    | 0.0  | 0.0     |

* excluded patients who have had hysterectomy without malignancy

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Fig 1. The cumulative incidence of malignant tumors in Lynch syndrome patients. (A) colorectal cancer, (B) endometrial cancer and (C) gastric cancer.

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−65) and 44 (range: 26–66) years, respectively. There were no significant differences between females and males regarding the age at which the first malignant tumor and the first LS-related tumor developed.

Table 4 shows the age-specific cumulative incidence of colorectal cancer, endometrial cancer, gastric cancer, small intestinal cancer, and renal/urinary tract cancer in LS patients. At the age of 50, the cumulative incidence was 50.9% (Fig 1A) for colorectal cancer, 17.4% (Fig 1B) for endometrial cancer, and 5.5% (Fig 1C) for gastric cancer. The cumulative colorectal cancer incidence tended to be higher in males than in females across all eligible patients ($P = 0.054$).

The cumulative colorectal cancer incidence in males was significantly higher than that in females in $MLH1$-mutated patients ($P = 0.02$) (Fig 2), while there were no significant differences in $MSH2$- and $MSH6$-mutated LS patients ($P = 0.70$ and $P = 1.00$, respectively).

In Table 5, the results of immunohistochemical staining for gastric cancer and non-LS-related tumors are presented. In gastric cancer, all 8 patients we treated in our hospital...
demonstrated loss of expressions of the MMR proteins. We treated 3 of 4 breast cancer patients, one of whom demonstrated a loss of expression of the MMR protein (Fig 3A). Additionally, 5 bladder cancer patients (Fig 3B) and 1 prostate cancer patient (Fig 3C) demonstrated loss of expression of the MMR protein. However, patients with thyroid cancer, spindle cell sarcoma, and giant cell tumors did not demonstrate a loss of expression of the MMR protein.

Discussion

In this study, we demonstrated tumor development in Japanese LS patients. These patients developed not only LS-related tumors but also non-LS-related tumors. In previous reports, the cumulative incidence at the age of 70 years was 54–74% for colorectal cancer, 28–60% for endometrial cancer, 5.8–13% for gastric cancer, 6.1–13.5% for ovarian cancer, 2.5–4.3% for small bowel cancer, 1.4–2.0% for biliary tract cancer, 0.4–3.7% for pancreatic cancer, 3.2–8.4% for ureter/renal pelvic cancer, and 2.1–3.7% for brain cancer [7–13]. Cancer risk has been reported to be different among MMR gene mutation carriers [15, 16]. The current study demonstrated the risk of various tumors, such as colorectal, endometrial, gastric, small bowel, and
ureter/urinal pelvic cancer, in LS patients. In all tumors except gastric cancer, tumor risks were similar to those reported in past reports [11, 12, 17]. However, the cumulative incidence of gastric cancer was higher in Japan than in Western countries, which was similar to the result in a previous report from Japan [18]. In East Asia, including Japan, gastric cancer is common in LS patients [18, 19]. It has been proposed that the development of gastric cancer is associated with *H. pylori* infection [20], which is common in Asia [21]. In the present study, all 8 LS patients with gastric cancer we treated had *H. pylori* infection and also had loss of expressions of the MMR proteins. Thus, these findings support the proposal that *H. pylori* infection increases the risk of gastric cancer in LS patients.

In the present study, we found non-LS-related tumors in LS patients: 6 patients with urinary bladder cancer, 4 with breast cancer, and 1 patient each with cervical cancer, thyroid cancer, prostate cancer, skin cancer, lymphoma, sarcoma, and bone cancer.

Recent reports have proposed that breast, urinary bladder [22], and prostate [23] cancers are also LS-related tumors. In the present study, we detected loss of expression of the MMR protein in 1 breast cancer patient, 5 urinary bladder cancer patients, and 1 prostate cancer patient. A report on breast cancer in LS patients by Lotsari *et al.* demonstrated that 65% of

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Fig 3. Immunohistochemical stainings for mismatch repair protein in malignant tumors in Lynch syndrome patients. (A) breast cancer with loss of MLH1 and PMS2 expressions, (B) bladder cancer with loss of MSH2 and MSH6 expressions and (C) prostate cancer with loss of MSH2 and MSH6 expressions.

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breast cancer tissues showed reduced or no MMR protein expression, corresponding to the germline mutation [24]. Gylling et al. reported that all 4 urinary bladder cancer patients with LS showed decreased MMR protein expression [25]. Moreover, a recent report has indicated that 70% of prostate cancer patients with LS demonstrated loss of expression of respective MMR proteins [26]. Therefore, these cancers are considered to be LS-related tumors in LS patients.

However, it is controversial whether thyroid cancer is an LS-related tumor. There are only few case report concerning thyroid cancer in LS [27–29]. The age-adjusted incidence of thyroid cancer was 21.0 per 100,000 women in the United States and 12.3 per 100,000 women in Japan, and the lifetime risk of developing thyroid cancer in women was 1.79% in the United States and 1.26% in Japan [30, 31]. In the present study, thyroid cancer patients did not show a loss of expression of the MMR protein. Thus, it is difficult to say that the incidence of thyroid cancer is high in LS patients because thyroid cancer developed in only 1 of the 55 LS patients.

The present study has the following limitations: (1) selection bias was caused by the retrospective nature, (2) treatment data were lacking, and (3) it was a single-center study. Nonetheless, considering that there are only a few published studies on LS-related cancers in Asia, we believe that our findings will help researchers and physicians clarify the nature of LS. However, further studies are required to overcome these limitations.

In conclusion, gastric cancer had a high incidence in Japanese patients with LS and was associated with \textit{H. pylori} infection. MMR protein deficiency causes malignant tumors to develop in breast, urinary bladder, and prostate tissues in LS patients.

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References

1. Bronner CE, Baker SM, Morrison PT, Warren G, Smith LG, Lescoe MK, et al. Mutation in the DNA mismatch repair gene homolog hMLH1 is associated with hereditary nonpolyposis colorectal cancer. Nature 1994; 368:258–61. https://doi.org/10.1038/368258a0 PMID: 8145827
2. Fishel R, Lescoe MK, Rao MR, Copeland NG, Jenkins NA, Garber J, et al. The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colorectal cancer. Cell 1993; 75:1027–38. PMID: 8252616

3. Miyaki M, Konishi M, Tanaka K, Kikuchi-Yanoshita R, Muraoka M, Yasuno M, et al. Germline mutation of MSH6 as the cause of hereditary nonpolyposis colorectal cancer. Nat Genet 1997; 17:271–2. https://doi.org/10.1038/ng1197-271 PMID: 9354786

4. Yamaguchi T, Iijima T, Mori T, Takahashi K, Matsumoto H, Miyamoto H, et al. Accumulation profile of frameshift mutations during development and progression of colorectal cancer from patients with hereditary nonpolyposis colorectal cancer. Dis Colon Rectum 2006; 49:399–406. https://doi.org/10.1007/s10350-005-0293-4 PMID: 16421660

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 Jun; 116(6):1453–6. PMID: 10348829

6. Umar A, Boland CR, Terdiman JP, Syngal S, de la Chapelle A, Ruschoff J, et al. Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. J Natl Cancer Inst 2004; 96: 261–268. PMID: 14970275

7. Dunlop MG, Farrington SM, Carothers AD, Kinzler KW, Vogelstein B: Cancer risk associated with germ-line DBA mismatch repair gene mutations. Hum Mol Genet 1997; 6: 105–110. PMID: 9002677

8. Barrow E, Robinson L, Alduaj W, Shenton A, Clancy T, Laloo F, et al. Cumulative lifetime incidence of extracolonic cancers in Lynch syndrome: a report of 121 families with proven mutations. Clin Genet 2009; 75: 141–149. https://doi.org/10.1111/j.1399-0004.2008.01125.x PMID: 19215248

9. Hampel H, Stephens JA, Pukkala E, Sankila R, Aaltonen LA, Mecklin JP, et al. Cancer risk in hereditary nonpolyposis colorectal cancer syndrome: later age of onset. Gastroenterology 2005; 129: 415–421. https://doi.org/10.1016/j.gastro.2005.05.011 PMID: 16083698

10. Stoffe E, Mukherjee B, Raymond VM, Tayob N, Kastrinos F, Sparr J, et al. Calculation of risk of colorectal and endometrial cancer among patients with Lynch syndrome. Gastroenterology 2009; 137: 1621–1627. https://doi.org/10.1053/j.gastro.2009.07.039 PMID: 19622357

11. Watson P, Vasen HF, Mecklin JP, Bernstein I, Aarnio M, Jarvinen HJ, et al. The risk of extra-colonic, extra-endometrial cancer in the Lynch syndrome. Int J Cancer 2008; 123: 444–449. https://doi.org/10.1002/ijc.23508 PMID: 18398828

12. 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; 89: 214–218. PMID: 10188721

13. Kastrinos F, Mukherjee B, Tayob N, Wang F, Sparr J, Raymond VM, et al. Risk of pancreatic cancer in families with Lynch syndrome. JAMA 2009; 302: 1790–1795. https://doi.org/10.1001/jama.2009.1529 PMID: 19861671

14. Kanda Y. Investigation of the freely available easy-to-use software ‘EZR’ for medical statistics. Bone Marrow Transplant 2013; 48:452–4. https://doi.org/10.1038/bmt.2012.244 PMID: 23208313

15. Bonadonna V, Bonaïti B, Olschwang S, Grandjouan S, Huiart L, Longy M, et al. French Cancer Genetics Network. Cancer risks associated with germline mutations in MLH1, MSH2, and MSH6 genes in Lynch syndrome. JAMA 2011; 305:2304–10. https://doi.org/10.1001/jama.2011.743 PMID: 21642682

16. Senter L, Ciendenning M, Sotamaa K, Hampel H, Green J, Potter JD, et al. The clinical phenotype of Lynch syndrome due to germ-line PMS2 mutations. Gastroenterology. 2008; 135:419–28. https://doi.org/10.1053/j.gastro.2008.04.026 PMID: 18602922

17. Capelle LG, Van Grieken NC, Lingsma HF, Steyerberg EW, Klokman WJ, Bruno MJ, et al. Risk and epidemiological time trends of gastric cancer in Lynch syndrome carriers in the Netherlands. Gastroenterology, 2010; 138:487–92. https://doi.org/10.1053/j.gastro.2009.10.051 PMID: 19900449

18. Yamaguchi T, Furukawa Y, Nakamura Y, Matsubara N, Ishikawa H, Arai M, et al. Comparison of clinical features between suspected familial colorectal cancer type X and Lynch syndrome in Japanese patients with colorectal cancer: a cross-sectional study conducted by the Japanese Society for Cancer of the Colon and Rectum. Jpn J Clin Oncol. 2015; 45:153–9. https://doi.org/10.1093/jjco/hyu190 PMID: 25404568

19. Park YJ, Shin KH, Park JG. Risk of gastric cancer in hereditary nonpolyposis colorectal cancer in Korea. Clin Cancer Res 2000; 6:2994–2998. PMID: 10955776

20. Huang JQ, Sridhar S, Chen Y, Hunt RH. Meta-analysis of the relationship between Helicobacter pylori seropositivity and gastric cancer. Gastroenterology. 1998; 114:1169–79. PMID: 9609753

21. Yamaoka Y. Mechanisms of disease: Helicobacter pylori virulence factors. Nat Rev Gastroenterol Hepatol. 2010; 7:629–41. https://doi.org/10.1038/nrgastro.2010.154 PMID: 20938460

22. Win AK, Young JP, Lindor NM, Tucker KM, Ahnen DJ, Young GP, et al. Colorectal and other cancer risks for carriers and noncarriers from families with a DNA mismatch repair gene mutation: A
prospective cohort study. J Clin Oncol 2012; 30: 958–964. https://doi.org/10.1200/JCO.2011.39.5590
PMID: 22331944

23. Raymond V, Mukherjee B, Wang F, Huang SC, Stoffel EM, Kastrinos F, et al. Elevated risk of prostate cancer among men Lynch syndrome. J Clin Oncol 2013; 14: 1713–1718.

24. Lotsari JE, Gylling A, Abdel-Rahman WM, Nieminen TT, Altimäki K, Friman M, et al. Breast carcinoma and Lynch syndrome: molecular analysis of tumors arising in mutation carriers, non-carriers, and sporadic cases. Breast Cancer Res. 2012; 14:R90. https://doi.org/10.1186/bcr3205 PMID: 22691310

25. Gylling AH, Nieminen TT, Abdel-Rahman WM, Nuorva K, Juhola M, Joensuu EI, et al. Differential cancer predisposition in Lynch syndrome: insights from molecular analysis of brain and urinary tract tumors. Carcinogenesis. 2008; 29:1351–9. https://doi.org/10.1093/carcin/bgn133 PMID: 18550572

26. Gylling AH, Nieminen TT, Abdel-Rahman WM, Nuorva K, Juhola M, Joensuu EI, et al. Differential cancer predisposition in Lynch syndrome: insights from molecular analysis of brain and urinary tract tumors. Carcinogenesis. 2008; 29:1351–9. https://doi.org/10.1093/carcin/bgn133 PMID: 18550572

27. Broaddus RR, Lynch PM, Lu KH, Luthra R, Michelson SJ. Unusual tumors associated with the hereditary nonpolyposis colorectal cancer syndrome. Mod Pathol 2004; 17: 981–989. https://doi.org/10.1038/modpathol.3800150 PMID: 15143336

28. Stulp R, Herkert A, Karrenbeld A, Sijmons RH. Thyroid cancer in Lynch syndrome. Case report and review of the expanding syndrome tumour spectrum. Hereditary cancer in clinical practice 2008; 6 (1):15–21. https://doi.org/10.1186/1897-4287-6-1-15 PMID: 19706203

29. Pelizzo MR, Pennelli G, Zane M, Galuppini F, Colletti PM, Merante Boschin I, et al. Papillary thyroid carcinoma (PTC) in Lynch syndrome: Report of two cases and discussion on Lynch syndrome behaviour and genetics. Biomed Pharmacother. 2015; 74:9–16. https://doi.org/10.1016/j.biopha.2015.06.008 PMID: 26349957

30. Howlader N, Noone AM, Krapcho M, Miller D, Bishop K, Kosary CL, et al. SEER Cancer Statistics Review, 1975–2013, National Cancer Institute. Bethesda, MD, http://seer.cancer.gov/csr/1975_2014/, based on November 2016 SEER data submission, posted to the SEER web site, April 2017.

31. The editorial board of the cancer statistics in Japan. Cancer statistics in Japan 2016, http://ganjoho.jp/data/reg_stat/statistics/brochure/2016/cancer_statistics_2016_app_E.pdf, based on March 2017