Original Article

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

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Abstract

Objective: The characteristics of familial colorectal cancer type X are poorly defined. Here we aimed to clarify the differences in clinical features between suspected familial colorectal cancer type X and Lynch syndrome in Japanese patients.

Methods: We performed germline mutation analyses of mismatch repair genes in 125 patients. Patients who met the Amsterdam Criteria I but lacked mismatch repair gene mutations were diagnosed with suspected familial colorectal cancer type X.

Results: We identified 69 patients with Lynch syndrome and 25 with suspected familial colorectal cancer type X. The frequencies of gastric and extracolonic Lynch syndrome-associated cancers were lower

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with suspected familial colorectal cancer type X than with Lynch syndrome. The number of organs with Lynch syndrome-associated cancer was significantly lower with suspected familial colorectal cancer type X than with Lynch syndrome. The cumulative incidence of extracolonic Lynch syndrome-associated cancer was significantly lower with suspected familial colorectal cancer type X than with Lynch syndrome. We estimated that the median cancer risk in 60-year-old patients with Lynch syndrome was 89, 36 and 24% for colorectal, endometrial and gastric cancers, respectively. Analyses of family members, including probands, revealed that the median age at diagnosis of extracolonic Lynch syndrome-associated cancer was significantly older with suspected familial colorectal cancer type X than with Lynch syndrome. The frequency of extracolonic Lynch syndrome-associated cancer was significantly lower with suspected familial colorectal cancer type X than with Lynch syndrome.

**Conclusion:** A significant difference in extracolonic Lynch syndrome-associated cancer was evident between suspected familial colorectal cancer type X and Lynch syndrome.

**Key words:** colorectal cancer, familial colorectal cancer type X, Lynch syndrome

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**Introduction**

A family history of colorectal cancer is found in ∼15% of all patients with the disease (1). Lynch syndrome (LS), formerly termed hereditary nonpolyposis colorectal cancer (HNPCC), is the most common familial colorectal cancer, and accounts for 1–3% of all colorectal cancer (2). LS with autosomal dominant inheritance is caused by germline mutations in mismatch repair (MMR) genes, including the MSH2 (3), MLH1 (4) and MSH6 (5) genes. Clinically, LS is characterized by high incidences of endometrial, small intestinal and urinary tract cancers, as well as an early-onset of colorectal cancer. Inactivation of MMR genes by germline and somatic mutations triggers LS-associated tumors. Consequently, a high frequency of replication errors results at both microsatellite regions and repetitive sequences in the coding regions of various growth-related target genes (6), creating a growth advantage for LS-associated tumors. However, some patients lack deficient mismatch repair (dMMR), regardless of a familial history of colorectal cancer. Recently, patients meeting the Amsterdam Criteria I (7) but lacking dMMR and/or microsatellite instability (MSI) in the tumor tissue were classified as having familial colorectal cancer type X (FCCTX), the cancer risk of which is lower than that of LS (8). Thus, the carcinogenesis of FCCTX is considered different from that of LS, because of the lack of dMMR. Even though the definition of FCCTX has not strictly been defined, but patients with dMMR in the germline but have no information about dMMR in the tumor tissue could be called ‘suspected FCCTX’. To date, neither FCCTX nor suspected FCCTX (s-FCCTX) cases have been reported in Japanese patients. To clarify the characteristics of s-FCCTX in Japanese patients, we compared the clinical features of s-FCCTX with those of LS. Despite mutation analyses, we were unable to detect MMR gene mutations in some patients. Therefore, we defined patients meeting the Amsterdam Criteria I but lacking MMR gene mutations as having s-FCCTX, and planned this study to clarify the differences in clinical features, particularly in incidence, between s-FCCTX and LS in Japanese patients with colorectal cancer.

The inclusion criteria were: age ≥16 years; histologically confirmed adenocarcinoma of the colon and rectum; invasion of the submucosa or deeper; and fulfillment of the modified Amsterdam Criteria II, which includes gastric cancer, because gastric cancer is common in Asian patients with LS (9). We also included first-degree relatives of the patient with germline MMR gene mutations. The modified criteria did not require pathological confirmation of a family history of cancer. The exclusion criteria were: familial adenomatous polyposis to be included in the Amsterdam Criteria II (10) as well as another polyposis syndrome: Cowden disease, Peutz-Jeghers syndrome and Juvenile polyposis syndrome.

After genetic counseling and the provision of written informed consent, the patients were enrolled in this study and given an anonymous identifier. Blood samples and clinical information were collected either from medical records or directly from patients using a case report form. Clinical information included: sex; date of birth; histological type; location of colorectal cancer; occurrence of extracolonic cancer; date at diagnosis of cancer, including extracolonic cancer; final confirmed surviving date; distinction between life and death; date of death; and family history. The study protocol and informed consent form were approved by the institutional review board at each institution.

**Patients and methods**

**Study design**

This nationwide cross-sectional study was conducted by the HNPCC Registry and Genetic Testing Project of the Japanese Society for Cancer of the Colon and Rectum (JSCCR) between September 2002 and July 2010. In this study, we aimed to clarify the following characteristics of Japanese patients with LS:

(i) the organ(s) in which the cancer developed;
(ii) the penetration rate of the cancer;
(iii) the prognosis of the cancer;
(iv) the relationship between genotype and phenotype; and
(v) the causative genes.

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**Follow-up survey**

In the follow-up survey, a case report form was sent annually to the data manager of each institution, requesting the anonymous identifier, occurrence of cancer, date at diagnosis of cancer; distinction between life and death; and family history. The study protocol and informed consent form were approved by the institutional review board at each institution.

**Mutation analysis**

Genomic DNA was extracted from peripheral blood samples using the standard phenol extraction/purification procedure. Mutation analysis was performed by direct sequencing of the entire coding region of MLH1, MSH2 and MSH6. The polymerase chain reaction (PCR) mixture (25 μl) contained 50 ng of genomic DNA, 0.4 μM of each...
primer, 1.5 mM MgCl₂, 0.2 mM of each NTP, 1× PCR buffer and 0.5 μl of KOD Plus DNA Polymerase (Toyobo Co., Ltd, Osaka, Japan). PCR comprised: initial denaturation at 95°C for 10 min; 35 cycles of denaturation at 95°C for 10 s; annealing at 57°C for 30 s; extension at 68°C for 1 min; and final extension at 68°C for 5 min. The PCR products were purified with ExoSAP-IT (Affymetrix Inc., Santa Clara, CA, USA) and underwent capillary sequencing using a BigDye® Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems Inc., Foster City, CA, USA) according to the manufacturer’s recommendations. The list of primer sequences used for PCR and sequencing are listed in Supplementary Table S1. The products were purified using a BigDye XTerminator Purification Kit (Applied Biosystems Inc.,) and loaded into a 3730xl Genetic Analyzer (Applied Biosystems Inc.).

For samples without deleterious mutations in the three genes, multiplex ligation of probe amplification was performed using a SALSA MLPA MLH1/MSH2 Kit (MRC-Holland, Amsterdam, The Netherlands) according to the supplier’s protocol (11). Because tumor tissue samples were not collected in this study, microsatellite instability was not analyzed.

Statistical analysis
The right colon comprises the cecum, ascending colon and transverse colon. We endeavored to collect clinical information from all patients; however, the pathological type of eight of 94 patients remains unknown.

Data are presented as totals, medians (range), means (95% confidence intervals) or percentages (95% CIs). Statistical analysis was performed using Fisher’s exact test and the Mann–Whitney U-test. Cumulative cancer risks were calculated using the Kaplan–Meier method, and the log-rank test was used to compare risks between s-FCCTX and LS. Statistical significance was defined as P < 0.05. All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan; http://www.jichi.ac.jp/saitama-sc/HP.files/statmedEN.html), a graphical user interface for R version 3.1.0 (The R Foundation for Statistical Computing, Vienna, Austria) (12). This interface is a modified version of R Commander version 2.0–4, which was designed to add statistical functions frequently used in biostatistics.

Results
Patients
Between September 2002 and July 2010, 142 patients were registered to the HNPCC Registry and Genetic Testing Project of the JSCCR with suspected LS. Of them, 123 were eligible for inclusion in this study, and 19 did not meet the inclusion criteria. However, we performed MMR gene mutation analyses on patients who did not meet the eligibility criteria: seven patients with a germline MMR gene mutation were included with the patients with LS. Among 142 patients, 69 met the Amsterdam Criteria I and 73 did not meet the Amsterdam Criteria I. Mutation analyses on the 137 patient’s enrolled detected 69 patients with germline MMR gene mutations. Of them, 36 had MLH1 mutations, 31 had MSH2 mutations and 2 had MSH6 mutations. Of the 142 patients, 25 were classified as having s-FCCTX, because they fulfilled the Amsterdam Criteria I without dMMR. We compared the 25 patients with s-FCCTX with the 69 patients with LS (Fig. 1).

Differences in characteristics between patients with s-FCCTX and patients with LS patients (proband)
Clinical characteristics are summarized in Table 1. The analyses of probands identified no significant difference in age at diagnosis of
The frequency of gastric cancer in patients with s-FCCTX was significantly lower than that in patients with LS ($P = 0.033$), and the frequency of endometrial cancer in patients with s-FCCTX tended to be lower than that in patients with LS ($P = 0.086$). The frequency of extracolonic LS-associated cancer in patients with s-FCCTX was significantly lower than that in patients with LS ($P = 0.001$). Whereas the median number of organs with LS-associated cancer was one for both patients with s-FCCTX and patients with LS, there was a significant difference between patients with s-FCCTX and patients with LS ($P = 0.001$). The mean number of organs with LS-associated cancer were $1.04 (0.96–1.12)$ in patients with s-FCCTX and $1.43 (1.27–1.60)$ in patients with LS ($P = 0.003$; Mann–Whitney U-test).

The cumulative extracolonic LS-associated cancer and colorectal cancer risks in patients with s-FCCTX were similar to those in patients with LS; however, the cumulative extracolonic LS-associated, endometrial and gastric cancer risks were significantly lower in patients with s-FCCTX than in patients with LS (Fig. 2). In this study, we estimated that the median cancer risk of 60-year-old patients with LS was $86\% (77–93\%)$ for colorectal cancer, $36\% (20–59\%)$ for endometrial cancer and $28\% (16–46\%)$ for gastric cancer; however, we did not estimate cancer risk in patients with s-FCCTX, because few extracolonic cancers occur.

Differences in characteristics between family members with s-FCCTX and LS

In the analyses of family members, including probands, the median age at diagnosis of LS-associated cancer was significantly greater in family members of patients with s-FCCTX than in family members of patients with LS ($P = 0.043$). When colon cancer was excluded, the tendency was stronger ($P = 0.007$): median age at diagnosis of colorectal cancer in family members of patients with s-FCCTX was similar to that in family members of patients with LS.

The frequencies of gastric cancer ($P = 0.003$) and endometrial cancer ($P = 0.001$) in family members of patients with s-FCCTX were significantly lower than those in family members of patients with LS. Moreover, the frequency of extracolonic LS-associated cancer in family members of patients with s-FCCTX was significantly lower than that in family members of patients with LS ($P < 0.001$).

**Discussion**

We defined patients meeting the Amsterdam Criteria I but lacking MMR gene mutations as having s-FCCTX. To date, the definition of FCCTX is still controversial. Originally, FCCTX collectively describes cases of colorectal cancer that meet clinical Amsterdam Criteria I, but whose tumors are DNA MMR proficient as assessed by MSI testing (8), however, some studies of FCCTX have also included Amsterdam Criteria II families with MMR-stable tumors (13–16). The study by Nieminen et al. (17) was based on FCCX families, which fulfilled the Amsterdam Criteria I/II or the Bethesda criteria without DNA MMR defects in tumor tissue or in the germline. Moreover, there is the problem with MSI testing. It has been reported that frequency of MSI-H is $69\%$ in LS with MSH6 mutation (18). Therefore, LS with MSH6 mutation may be included in the patients with Amsterdam Criteria who does not show MSI. In addition, the term ‘Lynch-like syndrome’ has recently been proposed (19). This indicates that dMMR (without hypermethylation of MLH1 promoter) without germline mutation of the MMR genes, a part of which may be

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**Table 1. Characteristics of suspected familial colorectal cancer type X and Lynch syndrome**

<table>
<thead>
<tr>
<th></th>
<th>s-FCCTX ($n = 25$)</th>
<th>LS ($n = 69$)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (proband) analysis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>16/25</td>
<td>33/69</td>
<td>0.24</td>
</tr>
<tr>
<td>Median age at diagnosis of colorectal cancer (range)</td>
<td>48 (21–74)</td>
<td>45 (24–70)</td>
<td>0.39</td>
</tr>
<tr>
<td>Median age at diagnosis of LS-associated cancer (range)</td>
<td>48 (21–74)</td>
<td>44 (24–70)</td>
<td>0.27</td>
</tr>
<tr>
<td>Median age at diagnosis of extracolonic LS-associated cancer (range)</td>
<td>54 (24–83)</td>
<td>52 (23–82)</td>
<td>0.36</td>
</tr>
<tr>
<td>Median number of colorectal cancer (range)</td>
<td>1 (1–3)</td>
<td>1 (1–4)</td>
<td>0.78</td>
</tr>
<tr>
<td>Right colon cancer</td>
<td>14/25</td>
<td>43/69</td>
<td>0.64</td>
</tr>
<tr>
<td>Poorly differentiated adenocarcinoma or mucinous carcinoma</td>
<td>6/24</td>
<td>15/62</td>
<td>$&gt;0.99$</td>
</tr>
<tr>
<td>Endometrial cancer</td>
<td>0/9</td>
<td>12/36</td>
<td>0.086</td>
</tr>
<tr>
<td>Small intestine cancer</td>
<td>1/25</td>
<td>4/69</td>
<td>$&gt;0.99$</td>
</tr>
<tr>
<td>Urinary tract cancer</td>
<td>0/25</td>
<td>7/69</td>
<td>0.18</td>
</tr>
<tr>
<td>Gastric cancer</td>
<td>0/25</td>
<td>11/69</td>
<td>0.033</td>
</tr>
<tr>
<td>Median number of LS-associated cancer organ (range)</td>
<td>1 (1–2)</td>
<td>1 (1–4)</td>
<td>0.003</td>
</tr>
<tr>
<td>Extracolonic LS-associated cancer</td>
<td>1/25</td>
<td>26/69</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Familial member analysis

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<table>
<thead>
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</thead>
<tbody>
<tr>
<td>Median number of first-degree relatives (range)</td>
<td>4 (2–13)</td>
<td>5 (2–12)</td>
<td>0.96</td>
</tr>
<tr>
<td>Median number of second-degree relatives (range)</td>
<td>1 (0–17)</td>
<td>1 (0–11)</td>
<td>0.31</td>
</tr>
<tr>
<td>Median age at diagnosis of colorectal cancer (range)</td>
<td>42 (21–55)</td>
<td>37 (22–60)</td>
<td>0.11</td>
</tr>
<tr>
<td>Median age at diagnosis of LS-associated cancer (range)</td>
<td>42 (21–55)</td>
<td>36 (20–60)</td>
<td>0.043</td>
</tr>
<tr>
<td>Median age at diagnosis of extracolonic LS-associated cancer (range)</td>
<td>67 (44–86)</td>
<td>49 (20–80)</td>
<td>0.007</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>25/25</td>
<td>64/69</td>
<td>0.32</td>
</tr>
<tr>
<td>Endometrial cancer</td>
<td>1/25</td>
<td>28/69</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Small intestine cancer</td>
<td>1/25</td>
<td>5/69</td>
<td>$&gt;0.99$</td>
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<td>0.18</td>
</tr>
<tr>
<td>Gastric cancer</td>
<td>7/25</td>
<td>43/69</td>
<td>0.005</td>
</tr>
<tr>
<td>Extracolonic LS-associated cancer</td>
<td>8/25</td>
<td>51/69</td>
<td>$&lt;0.001$</td>
</tr>
</tbody>
</table>

s-FCCTX, suspected familial colorectal cancer type X; LS, Lynch syndrome.
included in the category of ‘FCCTX’ because of a lack in examination of the tumor tissues. Therefore, we used ‘suspected FCCTX’ in this study.

The cancer risk of patients with LS was 40–80% for colorectal cancer, 25–60% for endometrial cancer and 1–4% for urinary tract cancer (20). However, whether gastric cancer is an LS-associated cancer remains controversial. In East Asia, it is believed that gastric cancer is common in patients with LS (10). Reportedly, European patients with LS lack the MMR protein corresponding to the germline mutation, and exhibit microsatellite instability (21). Our data demonstrated that gastric cancer is significantly more frequent with LS than with s-FCCTX. This finding suggests that gastric cancer is an LS-associated cancer.

With respect to cancer incidence, it was reported that only the incidence of colorectal cancer is higher, whereas the incidence of LS-associated cancer is lower, in patients with FCCTX than in patients with LS (8). This study showed that the frequencies of LS-associated cancers, including gastric cancer, were lower in patients with s-FCCTX than in patients with LS. These results are consistent with reports from Western countries. Moreover, the number of organs with LS-associated cancer was lower in patients with s-FCCTX than in patients with LS. These data support the theory that s-FCCTX is a completely different syndrome from LS.

The median age at diagnosis of cancer is reportedly 43–45 years in patients with LS (22). A difference in median age at diagnosis between patients with s-FCCTX and patients with LS is evident for every organ. For example, the median age at diagnosis of colorectal cancer was 47–50 years and that of endometrial cancer was 54 years (23), and the median age at diagnosis of colorectal cancer is lower in men than in women (24). Patients meeting the Amsterdam Criteria I with dMMR tend to develop colorectal cancer at a younger age than those meeting the Amsterdam Criteria I without dMMR (8). In our study, we demonstrated a later onset of LS-associated cancer in patients with s-FCCTX compared with that in patients with LS. However, the median age at diagnosis of cancer remained young with s-FCCTX compared with sporadic cases. These findings suggest that s-FCCTX is a hereditary syndrome. Gene alterations and expressions were different in FCCTX than in LS (13,25). Recently, studies to
identify the causative genes of FCCTX have been conducted, and some candidate genes have been proposed, such as CENPE, CDH18, GREM1, BCR, KIF24, GALNT12, ZNF367, HABP4, GABBR2 and BMP4 [26]. However, the causative genes of FCCTX have yet to be identified. In this study, additional investigation to detect causative gene of FCCTX was not performed yet. Further study is warranted.

This is first report of Japanese s-FCCTX patients. It is difficult to compare Japanese s-FCCTX patients to Western FCCTX patients directly. As mentioned above, however, both cancer incidence and median age at diagnosis of colorectal cancer in Japanese s-FCCTX patients were very similar to those in Western FCCTX patients.

The limitations of this study include low statistical power due to the limited number of cases of s-FCCTX (n = 25) and LS (n = 69), and the lack of data on PMS2 mutation. In this study, we did not analyze PMS2 mutation, because of the low frequency of PMS2 mutation and the number of pseudogenes of PMS2 [27]. However, we consider the influence of this on the overall results to be small. Nonetheless, considering that there are only a few publications on FCCTX and s-FCCTX, and none from Asia, we believe that our findings will help researchers and physicians clarify the nature of s-FCCTX.

In conclusion, our study indicated that, among Japanese patients with colorectal cancer, extracolonic LS-associated cancer occurred less frequently in patients with s-FCCTX than in patients with LS, the median age at diagnosis of extracolonic LS-associated cancer was greater in patients with s-FCCTX than in patients with LS, the number of organs with LS-associated cancer was lower in patients with s-FCCTX than in patients with LS, and the cumulative incidence of extracolonic LS-associated cancer was lower in patients with s-FCCTX than in patients with LS. A significant difference in extracolonic LS-associated cancer was evident between s-FCCTX and LS.

Conflict of interest statement
None declared.

References


