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<td>著者</td>
<td>伊藤 徹哉</td>
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Prevalence of Lynch syndrome among patients with upper urinary tract carcinoma in a Japanese hospital-based population

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Department of Gastrointestinal Tract Surgery, Fukushima Medical University School of Medicine, Fukushima, Japan.

Short running title:
Lynch syndrome in upper urinary tract cancer

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Abstract

Background: The prevalence of Lynch syndrome (LS) and use of universal tumor screening (UTS) to identify LS among unselected patients with upper urinary tract urothelial carcinoma (UUTUC), which is associated with LS, has not been closely investigated yet.

Methods: A total of 166 tumors from 164 UUTUC patients were tested for microsatellite instability (MSI) and expression of mismatch repair (MMR) proteins (MLH1, MHS2, MSH6, and PMS2) by immunohistochemistry (IHC). Genetic testing was performed for patients suspected of having LS. Clinicopathological factors, including familial and personal cancer history associated with mismatch repair deficiency, were evaluated.

Results: The frequency of high-level MSI (MSI-H) and loss of at least one MMR protein was 2.4% (4/164); the MSI and IHC results showed complete concordance. Of these four patients, three were genetically proven to have LS, while the remaining one was highly suggestive for LS based on their personal cancer history. Univariate analysis showed that age < 70 years old ($P = 0.04$), ureter as the tumor location ($P = 0.052$), previous history/synchronous diagnosis of colorectal cancer ($P < 0.01$), and fulfillment of the criteria per the revised Bethesda guideline ($P < 0.01$) tended to be or were significantly associated with MSI-H/ MMR loss.

Conclusions: The prevalence of LS among unselected UUTUC patients was at least 1.8% in our study population. The screening efficacies of the MSI test and IHC appear equivalent. UTS may be a valid approach; however, selective screening methods that consider factors associated with MMR loss/MSI-H tumors require further investigation.

Mini-abstract

We conducted universal tumor screening among 164 unselected upper urinary tract urothelial carcinoma patients. The prevalence of Lynch syndrome was estimated to be at least 1.8%.

Key words: Upper urinary tract urothelial carcinoma, Lynch syndrome, mismatch repair deficiency, immunohistochemistry, universal tumor screening, microsatellite instability
Introduction
Lynch syndrome (LS) is an autosomal dominant disease caused by germline pathogenic variant in DNA mismatch repair (MMR) genes (MLH1, MSH2, MSH6, PMS2) or a recently recognized deletion of the 3′ end of EPCAM, which is located upstream of MSH2 (1). LS carriers are prone to developing various malignant neoplasms such as colorectal, endometrial, upper urinary tract, gastric, small bowel, skin, brain, pancreatic, and bile duct cancers. Colorectal and endometrial cancers are most common (2), and upper urinary tract urothelial carcinoma (UUTUC) is the third most common in LS patients (3,4). The lifetime risk of developing UUTUC ranges from 3.2% to 6.0% (5-7), which is 7.6 to 22 times higher than in the general population (7-9).

Microsatellite instability (MSI), which is characterized by changes in repetitive DNA sequences, is a hallmark of deficient MMR tumors. The MSI test as well as detection of MMR proteins within tumors by immunohistochemistry (IHC) are widely used as screening methods to identify LS patients. It has been reported that the MSI test (MSI-H) and IHC (loss of at least one of MLH1, MSH2, MSH6, or PMS proteins) results are highly concordant, ranging from 92.4-97.8% in colorectal cancer (1, 10, 11) and 96.9-97.8% in endometrial cancer (10, 12). However, sufficient data on the concordance between the two different screening methods is lacking for UUTUC. This study was undertaken to investigate the prevalence of LS among unselected patients with UUTUC, to compare the results of both LS screening methods, and to evaluate the utility of UTS in UUTUC patients.

Patients and Methods
Ethical considerations
This study was conducted under the approval of the local ethics committee of the Saitama Medical Center (No. 924, No. 925, and No. 926) and the Saitama Medical University (No.592 and No. 747). Prior to the genetic testing for MMR gene mutation status, informed consent was obtained from the patients. For deceased cases, consent was obtained from their family members.

Patients
A total of 164 patients who had undergone total nephroureterectomy and found to have UUTUC by pathological examination at the Department of Urology, Saitama Medical Center, Saitama Medical University between March 2005 and November 2017 were enrolled in this study. Patient demographics, clinicopathologic data, and personal/family histories were
obtained from their medical charts. Tumor stage was determined according to the American Joint Committee on Cancer Staging Manual Seventh edition (13), and the grade was determined according to the 2004 World Health Organization (WHO) grading system (14).

**Immunohistochemistry (IHC) for DNA mismatch repair (MMR) proteins**

IHC was performed to detect 4 MMR proteins (MLH1, MSH2, MSH6, and PMS2) in 4-μm-thick formalin-fixed paraffin-embedded sections of tumors using a Staining Automat (BOND III, Leica Biosystems Melbourne Pvt. Ltd, Melbourne, Australia) according to the manufacturer’s protocol. The antibodies used for detecting MMR proteins were anti-hMLH1 antibody (clone G168-15, BIOCARE Medical LLC, Pacheco, CA, USA; 1:50), anti-hMSH2 antibody (clone FE11, BIOCARE Medical LLS, 1:50), anti-hMSH6 antibody (clone PU29, Leica Biosystems Newcastle Ltd, 1:70), and anti-hPMS2 antibody (clone M0R4G, Leica Biosystems Newcastle Ltd, 1:40 dilution).

Staining for MLH1, MSH2, MSH6, and PMS2 typically occurs in the nucleus. Thus, absence of nuclear staining in tumor cells accompanied by nuclear staining of non-neoplastic cells, such as normal urothelial cells and interstitial cells, was considered an abnormal pattern. Because MSH6 and PMS2 are obligate binding partners of MSH2 and MLH1, respectively, they are not expressed at the protein level in the absence of their partner proteins. Loss of both MSH2 and MSH6 expression may indicate alterations in **MSH2**. Loss of both MLH1 and PMS2 expression may indicate alterations in **MLH1**. Isolated loss of MSH6 or PMS2 expression may indicate alterations in **MSH6** or **PMS2**, respectively. whereas loss of PMS2 expression also indicates loss of MLH1. Loss of both MSH2 and MSH6 proteins with preservation of both of the relevant genes based on germline testing was considered to indicate a possible underlying **EPCAM** deletion.

**DNA and RNA extraction**

DNA was extracted from blood leukocytes or normal urothelial cells from formalin-fixed paraffin-embedded specimens using a QIAamp DNA Mini kit (Qiagen GmbH, Hilden, Germany). Total RNA was extracted from blood cells that had been cultured in the presence or absence of puromycin using an RNeasy Mini kit (Qiagen).

**Microsatellite instability (MSI) testing**

As previously described, an MSI test was conducted using five markers, namely BAT25, BAT26, D5S346, D2S123, and D17S250, in accordance with the revised Bethesda guidelines (15). After PCR amplification with specific primers, the fluorescent labeled products were
subjected to a fragment analysis using ABI 3130 or 3500 Genetic analyzer with a Gene Mapper software (Thermo Fisher Scientific Inc.). MSI results were recorded as microsatellite stable (MSS): no allele shift, MSI-L (low): one allele shift, or MSI-H (high): equal to or greater than two alleles. MSI was judged by comparing the matched cancerous and non-cancerous samples.

**Detection of germline mutations and copy number variances**

Sanger sequencing and/or Multiplex ligation-dependent probe amplification (MLPA) analyses were performed on DNA samples extracted from the formalin-fixed paraffin-embedded specimens using P072-C1 MSH6 probe mix (MRC-Holland, Amsterdam, Netherlands) to detect MMR genes based on the IHC results as previously described (16). For DNA samples obtained from blood cells, gene panel sequencing to detect hereditary gastrointestinal cancer syndrome was conducted as previously described (17). Candidate pathogenic variants were confirmed by a subsequent Sanger sequencing analysis. The pathogenicity of identified variants was confirmed based on the InSiGHT (https://databases.lovd.nl/shared/genes) and ClinVar databases (https://www.ncbi.nlm.nih.gov/clinvar/). For the novel variant, *MSH2:c.2635-3delC*, we conducted additional RNA sequencing analysis to assess its pathogenicity, as described elsewhere (18).

**Statistical analysis**

Data are expressed as medians and ranges, as appropriate. Categorical data were dichotomized where appropriate, and comparisons between groups were performed using the Fisher's exact probability test. Overall survival was defined as the time between operation and death by any cause. Survival times were compared using the log-rank test. *P*-values < 0.05 were considered to represent statistical significance.

**Results**

**Clinicopathologic characteristics**

The clinicopathologic characteristics of the patients enrolled in this study are shown in Table 1. There were 166 tumors from 164 patients who underwent surgery for UUTUC. If multiple lesions occurred only on the ipsilateral side, the largest (or larger) one was used as the representative for statistical description and analysis. The median age at diagnosis was 72 years (range, 39-88 years). There were 124 male patients (75.6%) and 40 female patients (24.4%). The main tumor location was the ureter in 79 patients (48.2%) and the renal pelvis in 85 patients (51.8%). Tumor histology indicated urothelial carcinoma in 162 cases (98.8%), small cell carcinoma in one case (0.06%), and squamous cell carcinoma in one case (0.06%). According
to the American Joint Committee on Cancer/International Union for Cancer Control staging system (13), 32 (19.3%) tumors were classified as Ta, four (2.4%) as Tis, 29 (17.5%) as T1, 26 (15.9%) as T2, 79 (42.1%) as T3, and 4 (2.4%) as T4. Based on the 2004 World Health Organization grading system (14), 45 (27.4%) were classified as low grade and 117 (71.3%) were classified as high grade. Twenty-five patients met the criteria under the revised Bethesda guidelines (19); 22 patients (13.4%) met one item, and three patients (1.8%) met three items of the 5 listed in the guidelines. No patients met the revised Amsterdam criteria (19). At the time of UUTUC diagnosis, nine patients developed colorectal cancer (n = 8) or had synchronous colorectal cancer (n = 1). Eight patients developed colorectal cancer secondary to UUTUC within the observation period of this study. Four patients (2.4%) developed bilateral tumors in both the renal pelvis and ureter; one patient developed them simultaneously while the other three patients developed them metachronously. For the metachronous multiple UUTUC patients, information on the metachronous lesion was described for statistical analysis when the initial lesion was outside the observation period, and the development of colorectal cancer was described in relation to the age at which UUTUC initially occurred.

**IHC for MMR protein detection and the MSI test**

IHC analysis indicated that among the 166 lesions, five lesions from four patients (2.4%) showed loss of at least one MMR protein. Loss of MSH6 was observed in one patient, and loss of MSH2 and MSH6 was observed in three patients (Figure 1, Figure 2). There were no patients showing loss of MLH1 or PMS2 expression. The MSI test was also performed for the 166 lesions. Five lesions from four patients (2.4%) showed MSI-H, three lesions from three patients (1.8%) showed MSI-L, and 160 lesions from 157 patients showed MSS (Figure 3). The MSI and IHC results showed complete concordance.

**Characteristics of the mismatch repair protein loss (MMRL)/MSI-H cases**

The comparison of the clinicopathologic factors between patients with MMRL/MSI-H and those with normal mismatch repair protein expression (NMMR)/MSS+MSI-L are shown in Table 3. The median age was 59 years (range, 50-66 years) for the MMRL/MSI-H group (n = 4), 72 years (range 39-88 years) for the NMMR/MSS+MSI-L group (n = 160), indicating a 13-year difference between the two groups (P = 0.02). The incidence of previous history or synchronous diagnosis of colorectal cancer was more frequent in the MMRL/MSI-H group than in the NMMR/MSS+MSI-L group (75.0% vs. 3.8%, respectively; P < 0.01). Fulfillment of the revised Bethesda guidelines was higher in the MMRL/MSI-H group than in the NMMR/MSS+MSI-L group (100% vs. 13.1%, respectively; P < 0.01). In regard to tumor
location, MMRL/MSI-H tumors were exclusively found in the ureter ($P = 0.052$). There were no differences pT in stage, grade, and presence or absence of multiple lesions between the two groups. Although patients in the MMRL/MSI-H group were all male, there were no statistically significant differences by gender ($P = 0.57$).

**Analysis of germline mutations**

A genetic test was performed for all four patients with MMRL/MSI-H. Three patients with loss of MSH2/MSH6 were diagnosed as having $MSH2$ germline pathogenic variants (Table 2). These variants were detected by Sanger sequencing or multigene panel testing using FFPE or blood samples. Case 1 and Case 2 showed the same single nucleotide mutation in $MSH2$ (NM 000251.2), c.942+3A>T, at the splicing donor site of intron 5; however, the two patients were considered to belong to unrelated families. Case 3 showed a single-base-pair deletion in $MSH2$, c.2635-3delC, affecting intron 15 splicing; the results of Case 3 were reported separately (18). For Case 4, no pathogenic variants were detected by Sanger sequencing. In addition, we were unable to obtain reliable results from the MLPA analysis of the $MSH6$ gene for Case 4 due to severe DNA degradation in the formalin-fixed paraffin-embedded specimens. Because Case 4 died, blood samples could not be analyzed. However, the individual also developed bladder cancer with isolated loss of $MSH6$ expression, suggesting LS development; his tumor located in the ascending colon was unavailable for MSI testing and MMR protein analysis.

**Discussion**

We have shown that: 1) the frequency of MMRL and MSI-H tumors among unselected UUTUC patients was 2.4%, and the results of two different screening methods were completely concordant; 2) the prevalence of LS was estimated to be at least 1.8% among these patients; and 3) univariate analysis showed that age < 70 years old ($P = 0.04$), previous history or synchronous diagnosis of colorectal cancer ($P < 0.01$), and fulfillment of either one of 5 items within the revised Bethesda guidelines ($P < 0.01$) were significantly associated with MMRL/MSI-H. In addition, MMRL/MSI-H tumors were exclusively developed in the ureter ($P = 0.052$).

There are a limited number of studies (20, 21) that have evaluated the efficacy of UTS for identification of LS among unselected UUTUC patients. To our knowledge, only two previous studies (21, 22) of universal tumor screening documented the results of genetic testing for MMR gene mutations. Ericson et al. (23) used 216 patients—the largest cohort reported. However, they did not perform genetic testing to identify LS. The present study is the second largest study to perform UTS among unselected UUTC patients; further, the present work is
notable in that two different screening methods were applied to the same samples, and that genetic testing was performed for LS among all candidates.

The previously reported frequencies of MMRL (MLH1, MSH2, MSH6, and PMS2) among unselected UUTUC patients ranged from 4.6% to 27.5% (20-24) by IHC (Table 4), which is greater than the number observed in the present study. The varying frequency of MMRL may largely depend on the different IHC methods, including the use of different antibodies, and/or the different methods used to discriminate between deficient MMR and proficient MMR tumors. In addition, race and geographic location of the study population in part may also affect the results. For example, the frequency of deficient MMR tumors and LS among unselected colorectal cancer patients differed between Western and Japanese populations (16). García-Tello et al. (24) reported an extremely high frequency of MMRL (27.5%). They noted that the frequency of PMS2 loss was the highest (95.5%) among all MMRL cases. This could not be explained by the undoubtedly low prevalence of PMS2 mutant carriers among LS-associated UUTUC patients. In addition, it is likely that weak staining of PMS2 was regarded as a deficient phenotype. In considering prior studies (but excluding the study by García-Tello et al.) the frequencies of MSH2/MSH6 loss and or the isolated loss of MSH6 among all cases of deficient MMR phenotypes ranged from 70.0-100%, which concur with the present study. The low frequency of MLH1/PMS2 loss seems notable in light of the results for patients with colorectal and endometrial cancer. The frequencies of MLH1/PMS2 loss among all cases of deficient MMR phenotypes ranged from 81.9-85.7% for colorectal cancer (16, 25, 26) and 65.2-81.3% (26-28) for endometrial cancer. The majority (67.8-96.2%) of tumors showing this IHC pattern was a consequence of epigenetic alteration, namely MLH1 promotor methylation (1, 10-12, 16, 29). Thus, the characteristic pattern of MMRL is concurrent with the relatively high prevalence of UUTUC in mutant MSH2 carriers among all LS families (6, 30-32) and the low prevalence (0.8%) of MLH1-methylated tumors among unselected UUTUC patients (33).

The reported frequencies of MSI-H tumors among the unselected UUTUC patients ranged from 3.0-31.3% (21, 23, 34), which was greater than that observed in the present study (Table 4). The frequencies of MSI-H might be affected by the different methods used to detect MSI, including the mononucleotide and dinucleotide markers and the criteria used to determine MSI-H status. Recently, the Pentalex™ and Promega™ panels comprising only mononucleotide repeat markers have been widely used. These panels have higher sensitivity for MSI-H compared with the Bethesda panel used herein, which comprises two mononucleotide and three dinucleotide repeat markers (35, 36). Most importantly, the results obtained by the MSI test and IHC in the present study showed complete concordance.
Sufficient data are lacking regarding the concordance rate of the MSI-H and MMRL for UUTUC, despite that recent studies have shown that the concordance rate reached 97.8% for colorectal cancers (10) and 96.9% for endometrial cancers (12). To our knowledge, in terms of UUTUC, the study by Ericson et al. (23) is the only one describing the concordance rate between two different screening methods. This study reported a concordance rate of 99.1%, which is concurrent with the present study. Discrepancies between the MSI test and IHC have been reported. For example, a missense variant of MLH1 with MSI-H status might demonstrate the presence of MLH1 in colorectal cancer cell nuclei (37). The prevalence of MLH1 loss seems lower in UUTUC than in colorectal cancers. Thus, the potential risk of discrepancy between the two universal tumor screening methods in terms of MLH1 missense variants might be minimized. Further investigations with larger patient cohorts are needed to conclude the two methods are similar in their abilities to detect LS in unselected UUTUC patients.

The prevalence of confirmed LS was 1.8% (3/164). The remaining one patient, whose formalin-fixed paraffin-embedded sample was not suitable for detection of germline MMR alterations, exhibited evidence suggesting that the patient was positive for LS. Specifically, the individual’s urinary bladder cancer showed isolated MSH6 loss by IHC; however, his colon cancer sample was not available for IHC analysis. The prevalence of LS as measured by UTS methods has not been closely investigated. Ju et al. (20) estimated that the prevalence ranges from 1-3% based on two recently published studies (21,22). However, genetic testing was performed for only some of the LS candidates. The results of the present study appear to concur with these previous estimations. Notably, the proportion of confirmed LS cases among the UUTUC patients with MMRL/MSI-H was higher than that observed for patients with colorectal or endometrial cancers with MMRL/MSI-H (2, 11, 16, 27).

Sufficient data on the effectiveness of UTS methods for patients with UUTUC is lacking. Implementation of UTS methods invariably raises questions regarding their cost-effectiveness. Work on LS screening methods in unselected patients with colorectal cancer has demonstrated that life-years gained and cost savings due to the prevention of additional cancers offset the cost of screening (38). Given the rarity of UUTUC relative to colorectal cancer, the costs associated with implementing UTS are anticipated to be relatively minor. Ju et al. (20) proposed that UTS of patients with UUTUC can be implemented in a cost-effective manner for the benefit of both LS patients and their blood relatives.

The use of a cutoff age offers a simple method for implementing selective screening for identification of LS. Specifically, ages < 70 years (39, 40) and ages < 60 years (41, 42) have been proposed for colorectal and endometrial cancer screening, respectively. Screening of patients < 60 years of age for UUTUC has been proposed by some investigators (43-45);
however, the majority of patients with MMRL/MSI-H in this study would have been overlooked using such an age cutoff. This result is in agreement with the results of Ju et al (20). No patients > 70 years old demonstrated MMRL/MSI-H in the present study. Thus, the age cut-off could be shifted to include individuals older than age 60; however, this change would require further investigation using a larger cohort.

Along with the age cutoff, tumor location, previous history/synchronous diagnosis of colorectal cancer, and fulfillment of the criteria listed in the revised Bethesda guidelines appears to be an effective approach for determining which UUTUC patients should be screened for LS. In the present study, all four patients with MMRL/MSI-H developed tumors in the ureter ($P = 0.052$), which is in agreement with previous studies (46, 47). However, other studies demonstrated a predominance of cancers in the renal pelvis (20, 48). Previous history or synchronous diagnosis of colorectal cancer is significantly associated with MMRL/MSI-H ($P < 0.01$). The close association between MMRL/MSI-H UUTUC and colorectal cancer has been previously reported (20). Ju et al (20) proposed that UUTUC may be a sentinel cancer of LS owing to the similar presentation age for colorectal and UUTUC. In the present study, there were no UUTUC patients with MMRL/MSI-H whose diagnosis of UUTUC preceded the diagnosis of colorectal cancer. Furthermore, the median age of patients diagnosed with UUTUC was reported to be 62 years (46), which is older than the average age at colorectal cancer diagnosis (mid 40s) in LS patients (19). The revised Bethesda guidelines were widely implemented before UTS of colorectal cancer was proposed. However, the sensitivity of this LS diagnostic method ranges from 68-89%, which can result in a high proportion of false negative results (1, 39). Thus, the importance of the revised Bethesda guidelines is unclear in screening candidates for genetic testing of MMR gene status. Furthermore, the criteria seem unfamiliar to urologists.

Pembrolizumab, an anti-PD-I antibody, is widely used as a second line of treatment for patients with metastatic UUTUC (49). Notably, it has come to be used for various solid tumors with MMRL/MSI-H (50, 51). The relevance of the LS diagnosis and detection of MMRL/MSI-H for the treatment of patients with unselected UUTUC by pembrolizumab requires further investigation. Furthermore, it seems intriguing whether UUTUC patients with MMRL/MSI-H would have more favorable prognosis than those with NMMR/MSI-L+MSS. Although we had only a limited number of MMRL/MSI-H cases, the overall survival times were not significantly different between the groups. Prognosis of UUTUC patients with MMRL/MSI-H also deserves further investigations.

In summary, although this is a relatively small, single-institutional retrospective study, the results are consistent with conclusions drawn from previous studies with large cohorts.
Furthermore, we observed several important findings. The prevalence of LS among unselected UUTUC patients was estimated to be at least 1.8% in our study population. The screening efficacies of the MSI test and IHC seem to be equivalent. UTS may represent a valid approach; however, selective screening methods that consider factors associated with MMRL/MSI-H tumors require further investigation.

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Conflict of Interest statement
None declared.
References


42. Buchanan DD, Tan YY, Walsh MD, et al. Tumor mismatch repair immunohistochemistry and DNA MLH1 methylation testing of patients with endometrial cancer diagnosed at age


Figures

Figure 1.
Flowchart of UTS for LS by IHC for MMR proteins


Patients with UUTUC
166 lesions from 164 patients

Single lesion
145 patients

Multiple lesions
21 lesions from 19 patients

Immunohistochemical staining

NMMRs
142 patients

MMRLs
3 patients

NMMRs
20 lesions from 18 patients

MMRL
1 lesion from 1 patient

MSH2/MSH6
2 patients

MSH6
1 patient

MSH2/MSH6
1 lesion from 1 patient
Figure 2.
x200 IHC for MMR protein in UUTUC specimens of the case 2 in Table 2. Loss of expression of MSH2 and MSH6 proteins was observed.

IHC: immunohistochemistry, MMR: mismatch repair, UUTUC: upper urinary tract urothelial carcinoma
Figure 3.
Flowchart of UTS for LS by MSI testing


Patients with UUTUC
166 lesions from 164 patients

Single lesion
145 patients

MSS
139 patients

MSI-L
3 patients

MSI-H
3 patients

Multiple lesions
21 lesions from 19 patients

MSS
21 lesions from 18 patients

MSI-L
0 cases

MSI-H
1 lesion from 1 patient

MSI testing
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<td><strong>Sex</strong></td>
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<td><strong>Grade</strong></td>
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<td>Tis 4 (2.4%)</td>
<td>T1 29 (17.7%)</td>
<td>T2 26 (15.9%)</td>
<td>T3 69 (42.1%)</td>
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<td><strong>Fulfillment of the rAMC</strong></td>
<td></td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Combination with colorectal cancer</strong></td>
<td></td>
<td>17 (10.4%)</td>
<td>Preceding to UTC 8 (4.9%)</td>
<td>Synchronous 1 (0.6%)</td>
<td>Secondary to UTC 8 (4.9%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Multiple or metachronous cases</strong></td>
<td></td>
<td>Single 145 (88.4%)</td>
<td>Multiple Unilateral 15 (9.1%)</td>
<td>Bilateral Synchronous 1 (0.6%)</td>
<td>Metachronous 3 (1.8%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* : median (range)
Tumor stage was determined according to the American Joint Committee on Cancer Staging Manual Seventh edition, and the grade was determined according to the 2004 World Health Organization grading system.

Lesions straddling the ureter and the renal pelvis were assigned based on the site where the lesion primarily existed. For metachronous cases, age, pT stage, history, grade, and location was described for the diagnosis of the first cancer. For synchronous multiple cases, pT stage, history, grade, and location were described for the most advanced lesion.

rBG: revised Bethesda guidelines, rAMC: revised Amsterdam criteria
**Table 2**
Clinicopathologic characteristics and evaluation of germline alterations of MMR genes in four patients with MMRL and MSI-H

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Number of criteria fulfilling the rBG</th>
<th>Tumor location</th>
<th>pT Stage</th>
<th>Grade</th>
<th>Pattern of mismatch repair protein loss</th>
<th>MSI status</th>
<th>Sample type</th>
<th>Germline alterations of MMR genes</th>
<th>Class†</th>
<th>History of LS associated tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>Male</td>
<td>1</td>
<td>Ureter</td>
<td>T2</td>
<td>High</td>
<td>MSH2/MSH6</td>
<td>High (5/5)</td>
<td>FFPE</td>
<td><strong>MSH2</strong>:c.942+3A&gt;T</td>
<td>5</td>
<td>Rectal Ca. (54 y.o.), UTC(66 y.o.), Bladder Ca. (67 y.o.)</td>
</tr>
<tr>
<td>2</td>
<td>64</td>
<td>Male</td>
<td>3</td>
<td>Ureter</td>
<td>T2</td>
<td>High</td>
<td>MSH2/MSH6</td>
<td>High (4/5)</td>
<td>Blood</td>
<td><strong>MSH2</strong>:c.942+3A&gt;T</td>
<td>5</td>
<td>A/C Ca. (44 y.o.), Gastric Ca. (48 y.o.), T/C Ca. (54 y.o.), Rectal Ca. (58 y.o.)</td>
</tr>
<tr>
<td>3</td>
<td>62</td>
<td>Male</td>
<td>3</td>
<td>Ureter</td>
<td>T2</td>
<td>High</td>
<td>MSH2/MSH6</td>
<td>High (4/5)</td>
<td>Blood</td>
<td><strong>MSH2</strong>:c.2635-3delC #</td>
<td>5</td>
<td>Colorectal Ca. (60 y.o.), Bladder Ca. (72 y.o.)</td>
</tr>
<tr>
<td>4#</td>
<td>66</td>
<td>Male</td>
<td>1</td>
<td>Ureter</td>
<td>T2</td>
<td>High</td>
<td>MSH6</td>
<td>High (2/5)</td>
<td>FFPE</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

*This variant containing the single-base-pair deletion, c.2635-3delC, within the splice acceptor site of the *MSH2* gene was reported to be associated with a defect in intron 15 splicing*

# We strongly suspect this patient has LS based on the loss of MSH6 expression in UTC and bladder cancers, as well as the patient’s history of colorectal cancer. Colorectal cancer tissue was not available for analysis.

†Pathogenicity of the identified variants was confirmed based on the InSiGHT and ClinVar databases.

Table 3
Comparison of clinicopathologic factors and overall survival between patients with MMRL/MSI-H and those with NMMR/MSI-L+MSS of the UUTUC

<table>
<thead>
<tr>
<th></th>
<th>Total (n=164)</th>
<th>MMRL/MSI-H (n=4)</th>
<th>NMMR/MSI-L+MSS (n=160)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>40</td>
<td>0</td>
<td>40</td>
<td>1.00</td>
</tr>
<tr>
<td>Male</td>
<td>124</td>
<td>4</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥70</td>
<td>91</td>
<td>0</td>
<td>91</td>
<td>0.57</td>
</tr>
<tr>
<td>&lt;70</td>
<td>73</td>
<td>4</td>
<td>69</td>
<td>0.04</td>
</tr>
<tr>
<td>pT stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤1</td>
<td>65</td>
<td>0</td>
<td>65</td>
<td>0.15</td>
</tr>
<tr>
<td>≥2</td>
<td>99</td>
<td>4</td>
<td>95</td>
<td></td>
</tr>
<tr>
<td>Grade</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>45</td>
<td>0</td>
<td>45</td>
<td>0.58</td>
</tr>
<tr>
<td>High</td>
<td>119</td>
<td>4</td>
<td>115</td>
<td></td>
</tr>
<tr>
<td>Location</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renal pelvis</td>
<td>85</td>
<td>0</td>
<td>85</td>
<td>0.052</td>
</tr>
<tr>
<td>Ureter</td>
<td>79</td>
<td>4</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>Previous history of colorectal cancer / Synchronous colorectal cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>9</td>
<td>3</td>
<td>6</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>no</td>
<td>155</td>
<td>1</td>
<td>154</td>
<td></td>
</tr>
<tr>
<td>Multiple morbidity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>19</td>
<td>1</td>
<td>18</td>
<td>0.39</td>
</tr>
<tr>
<td>No</td>
<td>145</td>
<td>3</td>
<td>142</td>
<td></td>
</tr>
<tr>
<td>Fulfillment of rBG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>25</td>
<td>4</td>
<td>21</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>No</td>
<td>139</td>
<td>0</td>
<td>139</td>
<td></td>
</tr>
<tr>
<td>Overall survival (months)*</td>
<td>70.4 (14.7 - 97.5)</td>
<td>85.5 (0.7-142.5)</td>
<td>0.41</td>
<td></td>
</tr>
</tbody>
</table>

*Median (range)
Clinicopathologic factors were analyzed by Fisher's exact test and overall survival was analyzed by the log-rank test.

Table 4
Reports of universal tumor screening of UUTUC by IHC and/or MSI testing

<table>
<thead>
<tr>
<th>Author</th>
<th>Year of publication</th>
<th>Country</th>
<th>Total no. of cases</th>
<th>Frequency of MSI-H</th>
<th>Frequency of MSI-L</th>
<th>MSI markers</th>
<th>Antibodies of IHC</th>
<th>Loss IHC</th>
<th>Pattern of MMR protein loss</th>
<th>Mutation analysis (rate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ericson KM, et.al (23)</td>
<td>2005</td>
<td>Sweden</td>
<td>216</td>
<td>4.2% (9/216)</td>
<td>2% (5/216)</td>
<td>BAT25, BAT26, BAT34, BAT40, D2S123, D5S346</td>
<td>MLH1, MSH2, MSH6, PMS2</td>
<td>4.6% (10/216)</td>
<td>MLH1/PMS2: 2 cases</td>
<td>MSH2: 7 cases, MSH2: 1 case</td>
</tr>
<tr>
<td>Metcalfe MJ, et al (21)</td>
<td>2018</td>
<td>USA</td>
<td>115</td>
<td>6.0% (5/87)</td>
<td>ND</td>
<td>BAT25, BAT26, BAT40, D2S123, D5S346, D17S250, TGFBR20</td>
<td>MLH1, MSH2, MSH6, PMS2</td>
<td>11.3% (13/115)</td>
<td>MSH2/MSH6: 6 cases</td>
<td>MSH6: 7 cases</td>
</tr>
<tr>
<td>Blaszyk H, et.al (34)</td>
<td>2002</td>
<td>Germany</td>
<td>114</td>
<td>31.3% (21/67)</td>
<td>16.4% (11/67)</td>
<td>BAT25, BAT26, BAT40, D2S123, D5S346, D17S250</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Garcia-Tello A, et.al (24)</td>
<td>2014</td>
<td>Spain</td>
<td>80</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>MLH1, MSH2, MSH6, PMS2</td>
<td>27.5% (22/80)</td>
<td>MLH1: 1 case</td>
<td>MSH2/MSH6: 5 cases, MSH6: 1 case, MSH2/PMS2: 10 cases, PMS2: 11 cases</td>
</tr>
<tr>
<td>Urakami S, et al (22)</td>
<td>2017</td>
<td>Japan</td>
<td>143</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>MLH1, MSH2, MSH6, PMS2</td>
<td>5% (7/143)</td>
<td>MSH2/MSH6: 1 case, MSH6: 1 case, MLH1/PMS2: 1 case</td>
<td>LS: 2 cases (1.4%) done for 2 cases</td>
</tr>
<tr>
<td>Ju JY, et al (20)</td>
<td>2018</td>
<td>USA</td>
<td>117</td>
<td>ND</td>
<td>ND</td>
<td>BAT25, BAT26, NR-21, NR-24, MONO-27 (Done for dMMR cases)</td>
<td>MLH1, MSH2, MSH6, PMS2</td>
<td>8.5% (10/117)</td>
<td>MSH6: 8 cases</td>
<td>MSH2/MSH6: 1 case, MLH1/PMS2: 1 case</td>
</tr>
<tr>
<td>Current sturdy</td>
<td>2019</td>
<td>Japan</td>
<td>164</td>
<td>2.4% (4/164)</td>
<td>1.8% (3/164)</td>
<td>BAT25, BAT26, D17S250, D2S123, D5S346</td>
<td>MLH1, MSH2, MSH6, PMS2</td>
<td>2.4% (4/164)</td>
<td>MSH2: 3 cases</td>
<td>MSH6: 1 case</td>
</tr>
</tbody>
</table>

UUTUC: upper urinary tract urothelial carcinoma, IHC: immunohistochemistry, MSI: microsatellite instability, MSI-H: high level instability, MSI-L: low level instability, LS: Lynch syndrome, ND: not detected