

# Upper tract urothelial carcinomas: frequency of association with mismatch repair protein loss and lynch syndrome

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Increased risk for upper tract urothelial carcinoma is described in patients with Lynch syndrome, caused by germline mutations in mismatch repair genes. We aimed to identify the frequency of mismatch repair protein loss in upper tract urothelial carcinoma and its potential for identifying an association with Lynch syndrome. We queried our database to identify upper tract urothelial carcinomas. Patients were cross-referenced for history of colorectal carcinoma or other common Lynch syndrome-associated neoplasms to enrich for potential Lynch syndrome cases. Tumor histopathologic characteristics were reviewed and each case was analyzed for loss of mismatch repair proteins, MLH1, MSH2, MSH6, and PMS2, by immunohistochemistry. Of 444 patients with upper tract urothelial carcinoma, a subset of 215 (encompassing 30 with upper tract urothelial carcinoma and another common Lynch syndrome-associated neoplasm) was analyzed for loss of mismatch repair protein expression. Of 30 patients with Lynch syndrome-associated neoplasms, six had documented Lynch syndrome, including two with Muir–Torre syndrome. Mismatch repair protein loss was identified in 7% of total upper tract urothelial carcinomas and 30% of patients with Lynch syndrome-associated neoplasms (including all patients with Lynch syndrome/Muir–Torre syndrome). Of patients without history of Lynch syndrome-associated neoplasms, 5 of 184 (2.7%) had loss of mismatch repair protein expression. Twelve cases with mismatch repair protein loss demonstrated loss of MSH2 and MSH6, and 2 had isolated loss of MSH6. MLH1 and PMS2 expression were consistently retained. Although increased intratumoral lymphocytes, inverted growth, pushing tumor-stromal interface, and lack of nuclear pleomorphism were more commonly seen in cases with mismatch repair protein loss, only intratumoral lymphocytes and presence of pushing borders were statistically significant. MLH1 and PMS2 testing appear to have little utility in upper tract urothelial carcinoma; however, mismatch repair protein loss of MSH2 and/or MSH6 by immunohistochemistry seems relatively sensitive and specific for identifying patients with potential Lynch syndrome.

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Urothelial carcinoma is the fourth most common cancer occurring in males in the United States and is estimated to account for 4–5% of male cancer-related deaths.<sup>1</sup> However, urothelial carcinoma occurring in the upper urinary tract, that is, in the renal pelvicaliceal system and/or ureter, has an annual incidence of 1–2 cases per 100 000 people and

accounts for only about 5–7% of all urothelial carcinomas.<sup>2–4</sup> Compared with bladder urothelial carcinoma, patients with upper tract urothelial carcinoma tend to present with higher grade and higher stage disease, which portend a worse overall prognosis.<sup>5,6</sup> The five-year cancer specific survival of upper tract urothelial carcinoma is greater than 90% for stage Ta or T1 disease, < 60% for T2 or T3 disease, and less than 10% for patients with T4 or metastatic (N/M1) tumors.<sup>7,8</sup> Although some studies suggest that patients with low-risk disease can be managed conservatively,<sup>6,9,10</sup> most patients with upper tract urothelial carcinoma are treated with nephroureterectomy or segmental ureterectomy.

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Lynch syndrome is an autosomal dominant hereditary tumor syndrome caused by germline mutations in genes encoding for mismatch repair proteins, which lead to microsatellite instability and increased risk of multiple tumor types.<sup>11,12</sup> The mismatch repair complex is critical in the recognition and repair of nucleotide bases that have been erroneously inserted during DNA replication. Microsatellites are short, polymorphic tandem repeats dispersed throughout the genome that are particularly sensitive to mutability in patients with mismatch repair defects.<sup>13,14</sup> A germline mutation in one of the major mismatch repair genes—*MLH1*, *MSH2*, *MSH6*, *PMS2*, and *EPCAM*—can be identified in ~90% of all Lynch syndrome patients.<sup>15–17</sup> Mutations in *MSH2* account for 60% of cases and *MLH1* for 30%, whereas *MSH6* (5–8%) and *PMS2* make up much of the remainder.<sup>18</sup> More recent studies have identified mutations in the *EPCAM* gene, which lead to inactivation of *MSH2*,<sup>16,19–21</sup> or inversions in the *MSH2* gene<sup>22,23</sup> as the causative abnormality in some patients; however, the specific underlying mutation remains unknown in a small percentage of cases.<sup>16,24</sup> Carcinogenesis in these patients is thought to be due to an accumulation of somatic frameshift mutations within microsatellite regions of various genes controlling growth and apoptosis.<sup>15</sup> These target genes appear to be tissue-specific and may account for the spectrum of disease seen in Lynch syndrome patients.<sup>25–27</sup>

Lynch syndrome is the most common hereditary cause of colorectal carcinoma, accounting for 2–3% of all colorectal carcinomas.<sup>16</sup> Although colorectal carcinoma and endometrial carcinoma are the most frequently associated neoplasms, other common Lynch syndrome-associated neoplasms include gastric and small bowel carcinomas, hepatobiliary carcinomas, ovarian carcinomas, central nervous system neoplasms and urothelial carcinomas, specifically of the upper urinary tract.<sup>21,28</sup> Prostate and testicular germ cell tumors have also been rarely reported in Lynch syndrome kindreds.<sup>21,29</sup> Muir–Torre syndrome, which is considered a variant of Lynch syndrome, includes patients with sebaceous neoplasms or keratoacanthomas of the skin. The majority of Muir–Torre syndrome patients have *MSH2* mutations; colorectal carcinoma and upper tract urothelial carcinoma are the most common visceral malignancies identified in these patients.<sup>20,30,31</sup>

Upper tract urothelial carcinoma occurs in 5% of patients with Lynch syndrome, making it the third most common malignancy in this patient population.<sup>5,13,32</sup> Patients with Lynch syndrome have a lifetime risk of developing upper tract urothelial carcinoma of up to 20%,<sup>33</sup> with the highest risk among patients with *MSH2* mutations.<sup>17,28,33–35</sup> Lynch syndrome patients with upper tract urothelial carcinoma present at a younger age and often lack the typical risk factors associated with urothelial carcinoma in the general population.<sup>29,36</sup> Studies have linked inverted growth pattern and low tumor stage in upper tract

urothelial carcinoma to microsatellite instability,<sup>14,27,37</sup> but other tumor characteristics, such as histologic grade and location, have shown variable results. Data regarding the risk of bladder urothelial carcinoma in Lynch syndrome as a whole are conflicting;<sup>38,39</sup> however, patients with *MSH2* mutations are at particular risk for the development of urothelial carcinoma, including carcinoma arising in the bladder.<sup>17,33,40,41</sup>

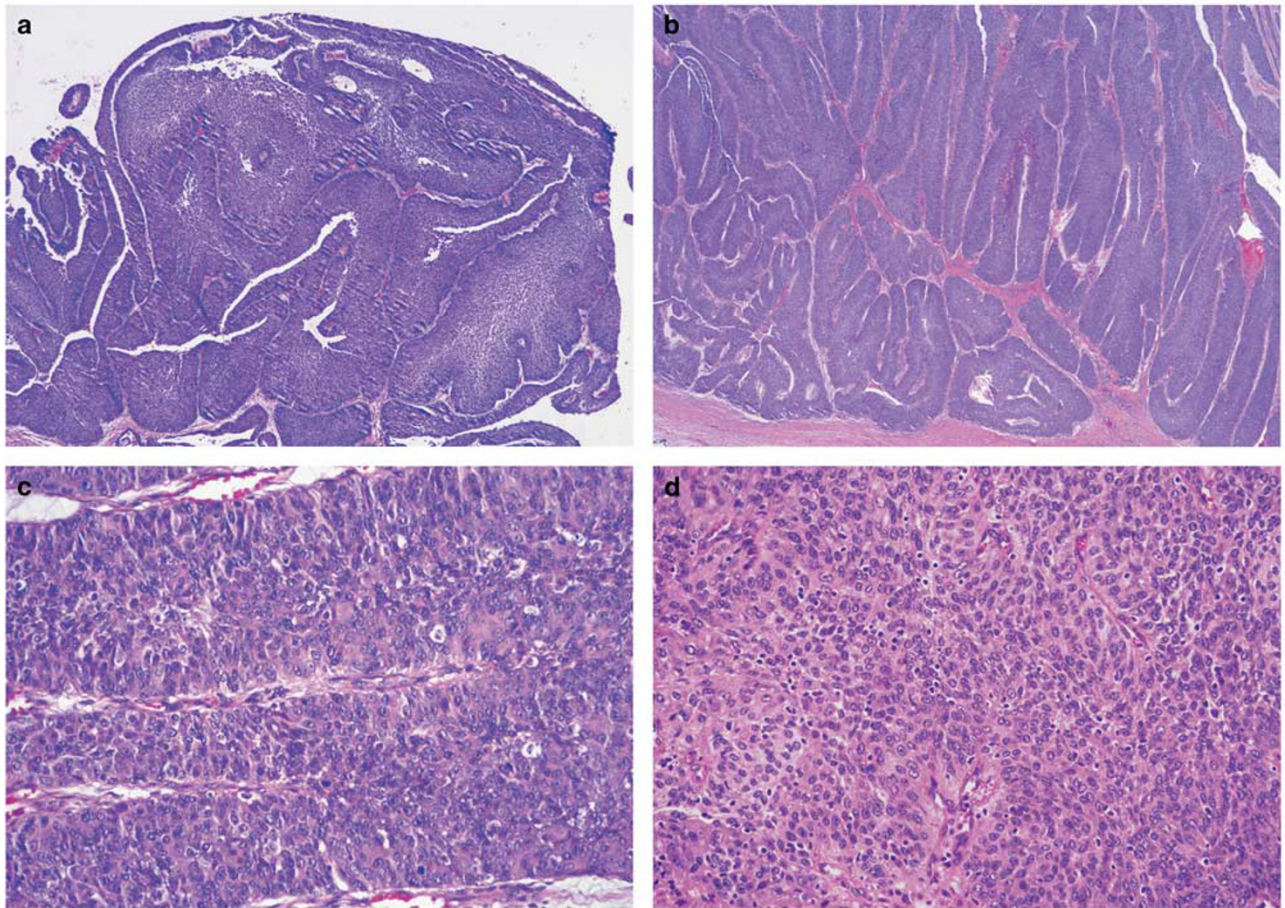
We evaluated the incidence of loss of mismatch repair protein expression by immunohistochemistry within a large cohort of upper tract urothelial carcinoma patients, enriched for cases with common Lynch syndrome-associated neoplasms (particularly colorectal carcinoma and endometrial carcinoma), to assess the utility of immunohistochemical studies targeting mismatch repair proteins in identifying patients with mismatch repair defects. As morphologic features characteristic of high microsatellite instability have been well documented in colorectal,<sup>16,42,43</sup> endometrial,<sup>44–46</sup> and ovarian carcinomas,<sup>47</sup> we also studied similar features to determine which upper tract urothelial carcinoma patients might benefit from additional mismatch repair testing.

## Materials and methods

A natural language search of the Cleveland Clinic pathology database (CoPath Plus) was performed to identify cases of upper tract urothelial carcinoma excised between 1995 and 2014. To maintain the purity of our series as papillary and/or invasive carcinomas arising within the upper tract, cases of flat carcinoma *in situ* and possible cases of involvement of the upper tract by a carcinoma of bladder origin were excluded. The patients' medical record numbers were cross-referenced with the Cleveland Clinic Lynch Syndrome Registry to identify study patients with a clinical diagnosis of Lynch syndrome. Medical record numbers were also cross-referenced with a second database of patients with colorectal adenocarcinoma (also retrieved via CoPath Plus natural language search), and all electronic medical charts were reviewed to identify patients with history of other common Lynch syndrome-associated neoplasms, particularly colorectal carcinoma or endometrial carcinoma, and Muir–Torre syndrome to enrich for potential Lynch syndrome patients.

For each case included in the study, all hematoxylin and eosin-stained (H&E) sections of tumor were reviewed and the following histopathologic characteristics recorded (Figure 1): histologic grade; growth pattern (papillary, papillary/endophytic, flat, solid/nodular); presence, type (pushing border vs infiltrative) and extent of invasion; presence, extent and composition of peri-tumoral inflammation; presence and extent of intratumoral lymphocytes; presence and extent of necrosis; presence of nuclear pleomorphism, apoptosis, and stromal desmoplasia.





**Figure 1** Common characteristics of tumors with mismatch repair protein loss by immunohistochemistry included inverted growth (a) or pushing borders without infiltration (b), high nuclear grade without significant pleomorphism (c), and moderate (2+) to marked (3+) intratumoral lymphocytic response (d).

Histologic grade of the non-invasive component was assigned based on the 2004 ISUP/WHO two-tiered grading scheme of low- and high-grade.<sup>48</sup> Occasional tumors showed predominantly low-grade morphology with focal high-grade areas accounting for < 5% of the tumor and were recorded as low-grade with focal high-grade features. Significant nuclear pleomorphism was defined as pleomorphism meeting the threshold of G3 in the WHO 1973 classification system.<sup>49</sup> Pushing border invasion was defined as a broad-based front with displacement of normal structures but without destructive infiltration of the tissue as nests or single cells. Peri-tumoral inflammation was considered 1+ if it was appreciable at low magnification but was restricted to the tumor base, 2+ if inflammation was also present within the papillary stalks of the tumor and 3+ if the inflammatory cells expanded the papillary stalks. Intratumoral lymphocytes were counted over 10 high power fields (hpf; field diameter of 0.55 mm) in areas of highest density. The extent of intratumoral lymphocytes was designated as 1+ (mild) if one to < 20 lymphocytes were present in 10 hpf, 2+ (moderate) if 20–29 lymphocytes were present and

3+(marked) if  $\geq 30$  lymphocytes were counted. As a formal scoring system for intratumoral lymphocytes for urothelial carcinoma has not been previously proposed in the literature, these points were arbitrarily assigned (with guidance from scoring systems utilized in endometrial carcinomas<sup>45</sup>) before scoring. True tumor cell necrosis was documented as 1+ if present in up to 5% of the tumor, 2+ if present in 6–50% and 3+ if present in > 50% of the tumor.

Four  $\mu\text{m}$ -thick tissue sections from a representative tumor block were subjected to immunohistochemical staining using anti-MLH-1 (clone G168.15, 1:20 in Van Gogh diluent; Biocare, Concord, CA, USA), anti-MSH-2 (clone FE11, 1:100 in Renoir Red Diluent; Biocare), anti-MSH-6 (clone BC/44, 1:100 in Renoir Red Diluent; Biocare), and anti-PMS-2 (clone A16-4, ready to use, Biocare) utilizing a Leica Bond Polymer Refine DAB detection system, as previously described.<sup>50</sup> Any degree of nuclear staining identified with appropriate staining of internal controls was considered positive. Loss of mismatch repair protein expression was defined by lack of nuclear staining in the lesional tissue, with appropriate internal controls. All H&E and

**Table 1** Summary of patients included in Lynch syndrome category

Pt <sup>a</sup>	Age	Gender	Common LAN	Other neoplasms	Germline status
2	53	F	CRC	Bladder UC	<i>MSH2</i> mutation: IVS5+3A>T
3	66	F	CRC, EC	LG NET	No personal genetic records Son has <i>MSH2</i> mutation: IVS5+3A>T
8	69	M	SebN	ParACA, Bladder UC	<i>MSH2</i> mutation: 420insT
10	90	M	CRC, AmpC	Bladder UC	No genetic records; UTUC and CRC tested in-study with loss of <i>MSH2/MSH6</i> in both
11	60	M	SebN	Bladder UC	No genetic records; Clinical MTS
13	57	M	CRC	Bladder UC	<i>MSH2</i> mutation: 421_422insT

Abbreviations: AmpC, ampullary carcinoma; CRC, colorectal carcinoma; EC, endometrial carcinoma; LAN, Lynch syndrome-associated neoplasm; LG NET, low-grade neuroendocrine tumor; MTS, Muir–Torre syndrome; ParACA, parotid adenocarcinoma; Pt, Patient; SebN, sebaceous neoplasms; UC, urothelial carcinoma; UTUC, upper tract urothelial carcinoma.

<sup>a</sup>Patient numbers correspond to those also listed in Table 3.

immunohistochemistry slides were reviewed by a single pathologist (HLH), with secondary review of select cases by at least one additional senior pathologist (CMG/TP). Review of morphology and immunohistochemistry results was carried out independently of each other and without knowledge of the patient's history.

## Results

Of the total 444 patients with upper tract urothelial carcinoma, we analyzed a subset of 215 tumors from 214 patients (one patient had synchronous bilateral upper tract urothelial carcinoma), which included 194 consecutive cases (spanning a period from 2009 to 2014) and 21 additional cases specifically selected due to patient history of Lynch syndrome or Lynch syndrome-associated neoplasm. Our study population included 144 males and 70 females (male to female ratio of ~2:1) with an average age of 70 years (range of 41–95 years). Thirty patients (14%) had history of at least one additional common Lynch syndrome-associated neoplasm, including 20 with colorectal carcinoma, 4 with endometrial carcinoma, 2 with colorectal carcinoma and endometrial carcinoma, 2 with sebaceous neoplasms, 1 with ovarian carcinoma, and 1 with cholangiocarcinoma. Six patients were included in the Lynch syndrome category for statistical analysis and are summarized in Table 1. Three of these patients (2 with colorectal carcinoma and bladder urothelial carcinoma and 1 with bladder urothelial carcinoma and multiple sebaceous neoplasms) had documented germline mutations in *MSH2* (c.IVS5+3A>T, c.421\_422insT, and c.420insT, respectively). A fourth patient was presumed to have Lynch syndrome as she has history of colorectal carcinoma, endometrial carcinoma and small bowel neuroendocrine tumor and her son has Lynch syndrome with a documented germline mutation in *MSH2* (c.IVS5+3A>T). A fifth patient with multiple sebaceous neoplasms had a history of Muir–Torre syndrome/Lynch syndrome,

however documented only clinically in our medical record system. Finally, one additional patient was highly suspicious for having Lynch syndrome, as both his upper tract urothelial carcinoma and colorectal carcinoma demonstrated loss of *MSH2/MSH6* by immunohistochemistry (both tested in-study). This patient also had a history of ampullary adenocarcinoma (no mismatch repair testing performed) and was included in the Lynch syndrome category (Table 1).

Eighty-four (39%) upper tract urothelial carcinoma cases arose within the ureter, 101 (47%) within the renal pelvis, and 30 (14%) involved both locations. Papillary (predominantly exophytic) growth was noted in 78% of cases ( $n=168$ ), however 23 of these cases (11% of cases overall) demonstrated inverted (endophytic) features, at least focally. No tumor was purely inverted/endophytic. Sixteen percent ( $n=34$ ) of tumors showed solid/nodular architecture and 6% ( $n=13$ ) were flat lesions. Tumor grade was distributed as follows: 179 (83%) high-grade, 26 (12%) low-grade, and 10 (5%) low-grade with focal high-grade features; 61 (28%) tumors were stage Ta, 42 (20%) T1, 27 (13%) T2, 76 (35%) T3 and 9 (4%) stage T4 (Table 2). Necrosis was identified in 41% of cases (24% 1+, 14% 2+, and 3% 3+).

Loss of mismatch repair protein expression was identified in 14/215 (7%) tumors (Table 3): 8 (57%) involved the ureter, 4 (29%) the renal pelvis, and 2 (14%) involved both sites. Ten of these cases were identified among the non-selected group of 194 patients (5%), with the remaining four occurring within the enriched group of 21 patients (19%). The ages of patients with loss of mismatch repair protein expression ranged from 45 to 90 years (mean: 64); 9 were male, and 5 female. Twelve (86%) cases with mismatch repair protein loss demonstrated loss of *MSH2* and *MSH6* (Figure 2), and 2 (14%) showed isolated loss of *MSH6* (Figure 3). All cases retained *MLH1* and *PMS2* expression. All cases with mismatch repair protein loss were high-grade (sensitivity = 100%; specificity = 18%; odds ratio = >999.999;  $P$ -value = 0.96) without variant histology



and 71% lacked nuclear pleomorphism (sensitivity = 71%; specificity = 56%; odds ratio = 3.1; *P*-value = 0.06; Figure 1c). Seven cases were pathologic stage Ta/T1 and 7 T2/T3. Twenty-one percent (3/14) of cases with mismatch repair protein loss displayed inverted growth features (Figure 1a), at least focally, compared with 10% (20/201) of cases with preserved

**Table 2** Selected histopathologic characteristics of all tumors by anatomic site

Tumor characteristic	UUC n = 84	U+ RPUC n = 30	RPUC n = 101	Total n = 215 (%)
<i>Grade</i>				
HG	67	29	83	179 (83)
LG	14	0	12	26 (12)
LG/HG	3	1	6	10 (5)
<i>Stage</i>				
Ta	25	3	33	61 (28)
T1	12	7	23	42 (20)
T2	16	2	9	27 (13)
T3	30	17	29	76 (35)
T4	1	1	7	9 (4)
<i>Growth pattern</i>				
Papillary				
Total	59	25	84	168 (78)
Exophytic	49	25	71	145 (67)
Inverted (focal)	10	0	13	23 (11)
Solid/nodule	19	3	12	34 (16)
Flat	6	2	5	13 (6)
Mismatch repair protein-IHC loss	8	2	4	14 (7)

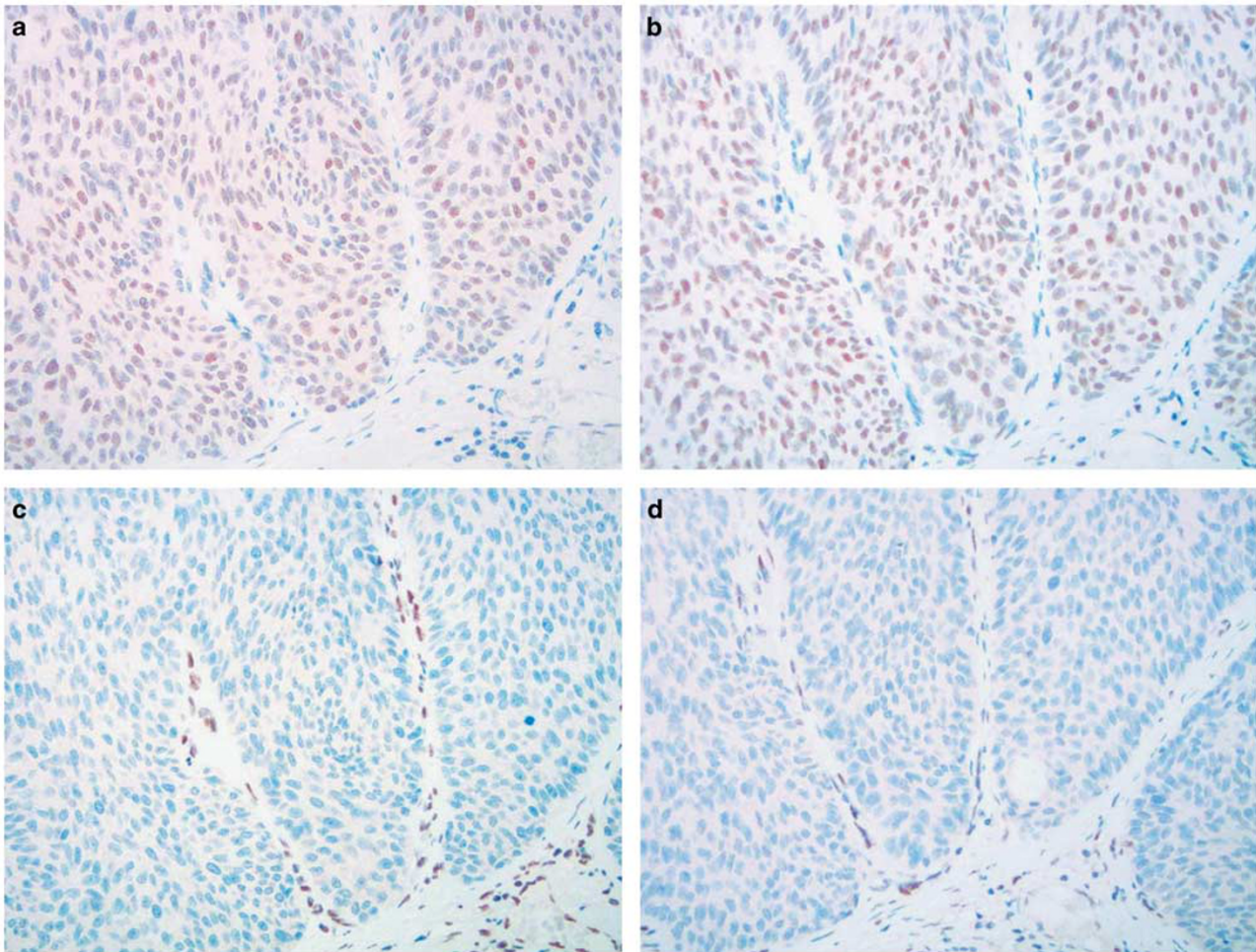
Abbreviations: HG, high-grade; LG, low-grade; LH/HG, low-grade with focal high-grade; RPUC, renal pelvis urothelial carcinoma; U+RPUC, ureteral plus renal pelvis urothelial carcinoma; UUC, ureteral urothelial carcinoma.

mismatch repair protein expression (sensitivity = 21%; specificity = 90%; odds ratio = 2.47; *P*-value = 0.19). Eighty-six percent of upper tract urothelial carcinoma with mismatch repair protein loss demonstrated pushing borders without destructive infiltrative edges (Figure 1b) compared to 53% of the cases with retained mismatch repair protein expression, a characteristic reaching statistical significance with a *P*-value of 0.03 (sensitivity = 86%; specificity = 47%; odds ratio = 5.4). Utilizing ROC curve analysis of intratumoral lymphocyte counts, a cut-point of 2+ ( $\geq 20$  lymphocytes/10 hpf) demonstrated the best balance of sensitivity and specificity (36 and 91%, respectively) for evaluation and was statistically significant between the two groups with a *P*-value of 0.0045 (odds ratio = 5.65). Moderate to marked (2–3+) intratumoral lymphocytes (Figure 1d) were identified in 5 (36%) upper tract urothelial carcinomas with loss of mismatch repair protein expression, compared to only 9% (18 of 201) of the cases with retained expression. Nine (64%) of the 14 tumors with mismatch repair protein loss occurred in patients with other common Lynch syndrome-associated neoplasms, including the 6 patients with documented Lynch syndrome. Among the other three patients with history of Lynch syndrome-associated neoplasms, one patient had additional history of colorectal carcinoma and met Bethesda guidelines for suspicion of Lynch syndrome and two patients had history of endometrial carcinoma, however, none of these patients had available genetic records. Of the five patients without history of Lynch syndrome-associated neoplasm, one had a family history suspicious for inclusion under the Amsterdam criteria, having both a sister and daughter diagnosed with uterine carcinoma. However, the ages at diagnosis could not be identified to allow

**Table 3** Characteristics of all tumors with mismatch repair protein-IHC loss

Pt.	Site	Grade	Stage (pT)	Border	Pleo.	Inv.	ITL per 10 hpf	Mismatch repair protein lost by IHC	Common LAN	Other neoplasms
1	RP	HG	1	P	–	+	0	MSH2/MSH6	–	–
2	RP	HG	a	P	–	+	0	MSH2/MSH6	LS (CRC)	Bladder UC
3	RP	HG	2	P	–	–	0	MSH2/MSH6	LS (CRC, EC)	Small bowel LG NET
4	U	HG	2	P	–	–	23	MSH2/MSH6	–	SCC
5	U	HG	3	I	+	–	0	MSH2/MSH6	EC	–
6	U	HG	a	P	+	–	0	MSH6	–	Bladder UC
7	U+RP	HG	3	P	+	–	0	MSH2/MSH6	EC	–
8	U	HG	1	P	–	–	35	MSH2/MSH6	LS/MTS (SebN)	ParACA, Bladder UC
9	U+RP	HG	3	P	–	–	0	MSH2/MSH6	–	Bladder UC
10	U	HG	3	P	–	–	0	MSH2/MSH6	LS (CRC, AmpC)	Bladder UC
11	U	HG	a	P	–	+	3	MSH2/MSH6	LS/MTS (SebN)	Bladder UC
12	U	HG	3	I	+	–	31	MSH2/MSH6	CRC	–
13	U	HG	a	P	–	–	39	MSH2/MSH6	LS (CRC)	Bladder UC
14	RP	HG	1	P	–	–	35	MSH6	–	–

Abbreviations: a, stage pTa; AmpC, ampullary carcinoma; CRC, colorectal carcinoma; EC, endometrial carcinoma; HG, high-grade; I, infiltrative; Inv, inverted; ITL, intratumoral lymphocytes; LAN, Lynch syndrome-associated neoplasms; LG NET, low-grade neuroendocrine tumor; LS, Lynch syndrome; MTS, Muir–Torre syndrome; P, pushing; ParACA, parotid adenocarcinoma; Pleo, pleomorphism; RP, renal pelvis; SebN, sebaceous neoplasia; SCC, squamous cell carcinoma of skin; U, ureter; UC, urothelial carcinoma; U+RP, ureter and renal pelvis.



**Figure 2** Example immunohistochemical profile in mismatch repair-deficient case demonstrating loss of MSH2 (c), and MSH6 (d) expression and retained expression of MLH1 (a) and PMS2 (b) in a patient with Muir–Torre syndrome.

definitive inclusion. The four remaining cases (2% of unselected cases) with mismatch repair protein loss lacked documented history of any common Lynch syndrome-associated neoplasm and had no available genetic records.

All six upper tract urothelial carcinomas occurring in patients with Lynch syndrome demonstrated high-grade morphology, yet lacked significant pleomorphism and had pushing rather than infiltrative growth. Two cases demonstrated increased (at least moderate/2+) intratumoral lymphocytes (33%) and two had inverted growth (33%), at least focally (Table 3).

## Discussion

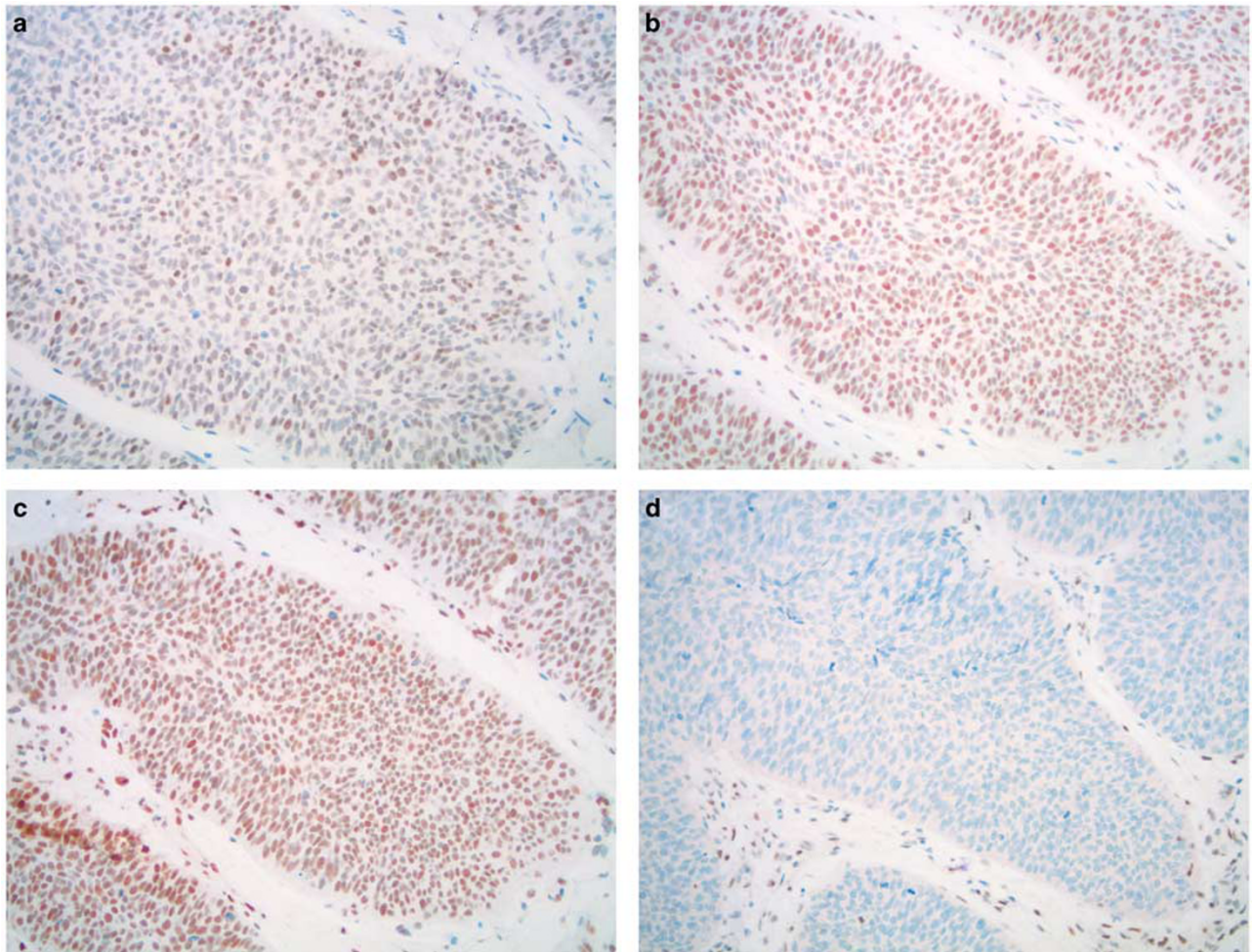
Urothelial carcinoma of the upper urinary tract is becoming increasingly recognized as an extracolonic manifestation of Lynch syndrome. Upper tract urothelial carcinoma occurs in 5% of patients with Lynch syndrome, making it the third most common malignancy in this patient pop-

ulation.<sup>5,13,32</sup> As such, there is a growing literature exploring this association.<sup>13,14,17,27,33–35,38,51–56</sup> To our knowledge, we report the largest series of predominantly unselected cases of upper tract urothelial carcinoma tested for mismatch repair protein loss by immunohistochemistry.

Lynch syndrome patients with upper tract urothelial carcinoma present at a younger age and often lack the typical risk factors associated with urothelial carcinoma in the general population.<sup>29,36</sup> In our study, patients with mismatch repair-deficient upper tract urothelial carcinoma had an average age of 64 years, compared with that of 70 years in the overall cohort. The majority of these lesions occurred in the ureter (57%), similar to findings reported by Hartmann *et al.*<sup>27</sup>

Patients suspected to have Lynch syndrome have historically been evaluated using the Amsterdam and Bethesda criteria;<sup>57–60</sup> however, these criteria may fail to identify many patients at risk of having Lynch syndrome.<sup>24</sup> As a result, pathology laboratories have been called on to improve detection through screening methodologies. In fact, universal





**Figure 3** Example immunohistochemical profile in mismatch repair-deficient case demonstrating isolated loss of MSH6 (d) expression and retained expression of MLH1 (a), PMS2 (b), and MSH2 (c) in a patient without additional history of common Lynch syndrome-associated neoplasm.

screening of all colorectal carcinoma<sup>61,62</sup> and endometrial carcinoma<sup>63–65</sup> cases has been proposed and implemented in many institutions. Screening for defects in the mismatch repair system is typically achieved through microsatellite instability testing via polymerase chain reaction or through immunohistochemical staining for mismatch repair proteins. Although there has been debate as to which of these methods is superior, it is generally recognized that these tests are complimentary and both may be necessary in certain cases.<sup>62,66,67</sup> However, both modalities are plagued by epigenetic silencing of mismatch repair genes via promoter hypermethylation, particularly of *MLH1*, in a significant number of sporadic cases (12% of upper tract urothelial carcinomas<sup>52</sup> and 10–15% of colorectal carcinoma<sup>16,24</sup>). Further evaluation using BRAF (for colorectal carcinoma) or methylation testing can help resolve which of these cases are truly sporadic and which may be due to a germline mutation.<sup>16</sup> More recent studies have also identified biallelic somatic mutations and loss of heterozygosity as the

cause of aberrant microsatellite instability and mismatch repair protein immunohistochemistry testing in a subset of patients,<sup>68–71</sup> highlighting the need for confirmatory germline mutation analysis in patients with apparent mismatch repair defects. In addition, specific cancer risks in patients with confirmed Lynch syndrome are strongly influenced by which mismatch repair gene is mutated,<sup>21</sup> so identification of the specific mutation should be sought to guide patient screening and follow-up, and to direct identification of additional affected family members.

Microsatellite instability was first described in upper tract urothelial carcinoma in 1998;<sup>29</sup> since then, high-level microsatellite instability has been documented in 4–31% of ‘sporadic’ upper tract urothelial carcinoma cases.<sup>27,38,51,54,55,72</sup> Having identified mismatch repair protein loss in 7% of the 215 upper tract urothelial carcinomas we evaluated, the incidence of a mismatch repair defect in our series is on the lower end of the reported incidence range. However, it is worth mentioning

that the majority of prior studies analyzed microsatellite instability using polymerase chain reaction with wide variability in the microsatellite markers utilized and in how the authors defined high microsatellite instability itself.<sup>29</sup>

Urothelial carcinoma is more likely to develop in patients with germline mutations in *MSH2*. In support of this notion, 86% of the cases demonstrating mismatch repair protein loss in our series lacked staining for both MSH2 and MSH6, suggesting an underlying *MSH2* mutation. The remaining 14% have a high likelihood of harboring a germline mutation in either *MSH6* or its partner, *MSH2*, as evidenced by loss of MSH6 protein expression. As prior studies have reported that immunohistochemical loss of MSH2 or MSH6 is frequently associated with an underlying germline mutation,<sup>16,20,24,37,73,74</sup> testing of upper tract urothelial carcinoma may prove to have higher yield of true mutations than colorectal carcinoma or endometrial carcinoma screening.

Interestingly, we identified mismatch repair protein loss by immunohistochemistry in 30% of the patients with common Lynch syndrome-associated neoplasms, and, in fact, the majority (64%) of patients with mismatch repair-deficient upper tract urothelial carcinoma had previous history of an additional Lynch syndrome-associated neoplasm, most commonly colorectal carcinoma. In addition, upper tract urothelial carcinoma from all six patients with Lynch syndrome demonstrated mismatch repair protein loss, suggesting that immunohistochemistry may be adequately sensitive for use in screening for mismatch repair defects in this population. Although all mismatch repair protein-negative cases showed loss of MSH2/MSH6 or MSH6, both of which are frequently associated with an underlying germline mutation,<sup>16,20,24,37,73,74</sup> it is more difficult to comment on the specificity of immunohistochemistry in our series as the germline status of all patients is not known.

Although it is less likely than colorectal carcinoma or endometrial carcinoma to be the sentinel lesion, upper tract urothelial carcinoma may be the presenting malignancy in a small number of Lynch syndrome patients. We identified loss of mismatch repair protein expression in 2.7% (5/184) of the patients without history of any additional Lynch syndrome-associated neoplasm. As such, identifying morphologic characteristics common to mismatch repair-deficient upper tract urothelial carcinoma may prove helpful in identifying patients without history of Lynch syndrome-associated neoplasms that may benefit from further testing. Several previous reports have suggested inverted growth pattern, pushing borders, and low tumor stage as features suggestive of mismatch repair deficiency.<sup>13,14,27,35</sup> Additional studies have examined the degree of inflammatory response<sup>14,27</sup> and tumor grade.<sup>13,14,27,34,35,38,51</sup> The results of these studies have been quite varied, and consistently reproducible morphologic features have not yet been defined. We also sought to identify unifying features

among our cases demonstrating mismatch repair protein loss by immunohistochemistry. Lack of nuclear pleomorphism (sensitivity = 71%; specificity = 56%; odds ratio = 3.1; *P*-value = 0.06), inverted growth pattern (sensitivity = 21%; specificity 90%; odds ratio = 2.47; *P*-value = 0.19) presence of pushing borders without destructive infiltrative edges (sensitivity = 86%; specificity = 47%; odds ratio = 5.4) and increased intratumoral lymphocytes (sensitivity = 36%; specificity = 91%; odds ratio = 5.65) emerged as features which may help identify potential Lynch syndrome candidates. Among these characteristics, increased intratumoral lymphocytes ( $\geq 20$  TIL/10 hpf) and pushing tumor/stromal interface reached statistical significance with *P*-values of 0.0045 and 0.03, respectively. It is clear that no one morphologic feature is sensitive or specific enough to suggest Lynch syndrome with certainty, but that these features may need to be considered as a constellation of findings when contemplating further analysis for mismatch repair defects.

To our knowledge, the findings reported herein represent the largest series of predominantly unselected upper tract urothelial carcinomas assessed for mismatch repair defects via immunohistochemistry. Our exploration of histopathologic features noted among mismatch repair-deficient tumors failed to identify any with the degree of sensitivity needed for screening, but does suggest that the presence of pushing borders and increased intratumoral lymphocytes should prompt consideration of additional testing. Our data also suggests that immunohistochemistry has sufficient sensitivity to serve as a screening tool for underlying mismatch repair defects. In fact, it appears as though limited assessment for MSH2 and MSH6 only may have more utility for screening in suspicious cases than assessment of all four mismatch repair proteins. We also provide additional evidence that we hope will help guide future discussions regarding the appropriateness of universal screening in upper tract urothelial carcinoma. Our data strongly suggest that special consideration for additional testing should at least be paid to younger patients and those with history of other common Lynch syndrome-associated neoplasms.

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## Disclosure/conflict of interest

Brandie Heald serves on the speakers' bureau for Myriad Genetics Lab and on the advisory board for Invitae. The remaining authors declare no conflict of interest.



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