original reports

Cancer Susceptibility Mutations in Patients With Urothelial Malignancies

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abstract

PURPOSE Urothelial cancers (UCs) have a substantial hereditary component, but, other than their association with Lynch syndrome, the contribution of genetic risk factors to UC pathogenesis has not been systematically defined. We sought to determine the prevalence of pathogenic/likely pathogenic (P/LP) germline variants in patients with UC and identify associated clinical factors.

PATIENTS AND METHODS Overall, 586 patients with UC underwent prospective, matched tumor-normal DNA sequencing. Seventy-seven genes associated with cancer predisposition were analyzed; allele frequencies were compared with publicly available database.

RESULTS P/LP germline variants were identified in 80 (14%) of 586 individuals with UC. The most common P/LP variants in high- or moderate-penetrance genes were *BRCA2* (n = 9; 1.5%), *MSH2* (n = 8; 1.4%), *BRCA1* (n = 8; 1.4%), *CHEK2* (n = 6; 1.0%), *ERCC3* (n = 4; 0.7%), and *NBN* and *RAD50* (n = 3; 0.5% each). Sixty-six patients (83%) had germline P/LP variants in DNA-damage repair (DDR) genes, of which 28 (42%) had biallelic inactivation. Patients with P/LP variants were more commonly diagnosed at an early age (22% *v* 6% in those without variants; $P = .01$). *BRCA2* and *MSH2* were significantly associated with an increased risk for UC (odds ratio, 3.7 [$P = .004$] and 4.6 [$P = .001$], respectively). Current clinical guidelines for referral for genetic testing failed to identify 6 (26%) patients with high-penetrance variants.

CONCLUSION Clinically significant P/LP germline variants in DDR genes frequently are present in patients with advanced UC. The presence of DDR germline variants could guide cancer screening for patients and their families and serve as predictive biomarkers of response to targeted or immunotherapies. Family history–based criteria to identify patients with hereditary UC susceptibility are insensitive. Broader germline testing in UC, particularly in those of young ages, should be considered.

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INTRODUCTION

Urothelial cancer (UC) has a substantial hereditary component, with an estimated 30% heritable fraction according to epidemiologic studies.[1] Family history of UC confers a twofold increased risk, with numerous reports of multiple-case UC kindreds.[2-6] The heritable mechanisms underlying familial aggregations and early-onset UC remain unknown, and there are few syndromic associations with known cancer susceptibility genes. Highly penetrant cancer susceptibility genes, such as those in the mismatch-repair (MMR) pathway, only account for a small fraction of inherited UC susceptibility; mutations in MMR-associated genes are found primarily in patients with tumors of the ureter and renal pelvis.[7,8]

Beyond their role in tumor pathogenesis, germline variants can be predictors of response to cancer therapies that have activities enhanced by DNA repair defects.[9,10] Deficient MMR status and high microsatellite instability (MSI-H), hallmarks of tumors in patients with Lynch syndrome, are predictive of response to the PD-1 inhibitor pembrolizumab.[11] In UC, inactivating somatic mutations in *ERCC2*, *ATM*, *RB1*, and *FANCC* have been associated with response to cisplatin-based neoadjuvant chemotherapy.[12,13] DNA-damage repair (DDR) mutations, both somatic and germline, may independently predict response to checkpoint inhibitors in patients with UC.[14] The substantial heritability of UC, the incomplete understanding of the genes and pathways responsible for this increased heritable risk, and the potential that identification of germline variants could help guide therapeutic decisions provide a strong rationale to investigate putative UC susceptibility genes.

We have shown that germline mutations are commonly identified in individuals undergoing tumor-normal next-generation sequencing (NGS) and may reveal

ASSOCIATED CONTENT

Appendix

Author affiliations and support information (if applicable) appear at the end of this article.

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previously unknown genetic associations.[15,16] Using a matched tumor-germline NGS platform, we determined the prevalence of pathogenic/likely pathogenic (P/LP) germline variants in 77 cancer-associated genes in patients with UC, and we examined associations between germline status and somatic mutational profile.

PATIENTS AND METHODS

Patient Cohort

Beginning in May 2014, patients with UC seen at Memorial Sloan Kettering Cancer Center were offered matched tumor-germline DNA sequencing at physician discretion under an institutional protocol (ClinicalTrials.gov identifier: NCT01775072), with only somatic variants reported. Baseline clinical characteristics for all enrolled patients were collected from institutional electronic medical records. Age $\leq$ 45 years was prospectively defined as early onset (outside the 95% CI of the median age of UC diagnosis).[17] Starting in May 2015, patients could opt in to receive results of a secondary germline analysis of genes associated with increased heritable cancer risk; 169 patients consented to disclosure of germline results. For these 169 patients (identified cohort), germline variants were associated with a broad range of clinical features. For the remaining 417 patients (anonymized cohort), analysis was performed in a permanently anonymized fashion with no clinical annotation beyond tumor subtype. The study was approved by the Memorial Sloan Kettering Cancer Center institutional review board.

Sequencing and Results Reporting

Paraffin-embedded tumor and blood from patients were obtained and sequenced using the MSK-IMPACT platform, a capture-based NGS assay capable of identifying mutations, copy number alterations, and select gene fusions involving 341 cancer-associated genes in the first iteration and 468 in the more recent iteration, as described previously (gene list; Appendix Table A1, online only).[18,19] For the anonymized cases, sequence data were assigned a unique study identifier and irretrievably de-linked from personal identifiers before variant calling.

Variant Interpretation

Pathogenicity was determined according to American College of Medical Genetics criteria (updated as of January 2018).[20] Germline variants in 77 cancer predisposition genes were prioritized using PathoMAN, an automated germline variant classification tool for cancer; variants from the Exome Aggregation Consortium (ExAC) database without The Cancer Genome Atlas (TCGA) alleles were similarly prioritized.[21] In addition, manual curation of the dataset was performed by a research genetic counselor (Y.K.), and any differences in variant calls were resolved with review by a molecular geneticist (O.C.B.) and a cancer geneticist (M.I.C.). For these 77 genes, all coding regions were sequenced in both the germline and the tumor. P/LP germline variants (associated with disease causation) were included in this analysis; variants of unknown significance were reviewed but were not reported. According to known disease risks and prior modeling, P/LP variants were classified at the gene level as high penetrance (relative risk [RR] of disease, >4), moderate penetrance (RR, 2-4), low-penetrance (RR, <2), or uncertain penetrance, or they were associated with an autosomal recessive condition.[22] For *CHEK2*, *APC*, and *ERCC3*, classification was performed at the variant level: *APC* p.Ile1307Lys and *CHEK2* p.Ile157Thr were considered low or uncertain penetrance, and the *ERCC3* p.Arg109X, moderate penetrance.[23] Tumor sequencing results were available for all patients. The FACETS algorithm was used to evaluate loss of heterozygosity (LOH) at the locus of the germline variant.[24]

We identified 34 genes within the MSK-IMPACT panel as related to DDR, as previously described (Appendix Table A2, online only).[25] Within DDR genes, *MSH2*, *MSH6*, *MLH1*, and *PMS2* were classified as MMR pathway genes; the remaining DDR genes were classified as other DDR.

Comparison of Guidelines Based Versus Agnostic Testing for Cancer Predisposition Syndromes in the Identified Cohort

Family history, religion, and race/ethnicity were self-reported and collected from the medical record or at time of genetic counseling. Published guidelines based on personal and family history were used to determine which genetic tests would be indicated according to the age at onset of cancer, personal or family history of cancer, and self-reported Ashkenazi Jewish ancestry.[26,27] A pathogenic variant was considered incremental if it was detected by sequencing in this study but would not have been identified by genetic testing through application of current clinical guidelines.

Statistical Analysis

Allele frequencies of P/LP variants in the identified and anonymized cohorts were compared with allele frequencies of P/LP variants in the ExAC without cases from TCGA, as described previously.[21] Ashkenazi Jewish founder mutations were excluded (Appendix Table A3, online only). Fisher's exact 2-sided test was performed to assess differences in frequency; odds ratios with 95% intervals were reported. The α used to determine statistical significance in the Fisher's test and the 95% confidence limits was adjusted using the Bonferroni correction for multiple comparisons. In the identified cohort, clinical characteristics of patients with germline P/LP variants were compared with those without them using Fisher's exact test. Statistical analysis was performed using SAS 9.4 (Cary, NC) and R version 3.3.3. Response to therapy in identified patients with MMR mutations treated with immunotherapy was assessed according to RECIST v1.1 criteria.

RESULTS

Patient Characteristics

Five hundred eighty-six patients had tumor-germline profiling using MSK-IMPACT; 417 consented to receive only tumor sequencing results (anonymized cohort), and 169 consented to receive both tumor and germline sequencing results (identified cohort). Clinical characteristics of patients are listed in Table 1. Patients were primarily men (74.1%) and had a median age of 63 years (range, 25-87 years); 42 (7.2%) were ≤ 45 years of age. Only 42 patients (7.2%) had a family history of bladder or UC, and 112 (19.1%) had a history of a second malignancy other than UC. Most patients (79.0%) had bladder as the primary tumor site, and 59.7% had or developed metastatic disease during the period of clinical follow-up.

Frequency and Spectrum of Germline Variants

Eighty-six P/LP variants were identified in 80 individuals (13.7%), including 62 patients (13.4%) with bladder and 18 (15.8%) with upper tract (UT) tumors (Fig 1). Eleven patients had two P/LP variants each. The most frequently mutated moderate- or high-penetrance genes were *BRCA2* (n = 9; 1.5%), *MSH2* (n = 8; 1.4%), *BRCA1* (n = 8; 1.4%), *CHEK2* (n = 6; 1.0%), *ERCC3* (n = 4; 0.7%), and *NBN* and *RAD50* (n = 3; 0.5% each; Appendix Table A4, online only). The low-penetrance variant *APC* p.Ile1307Lys was the most prevalent overall (n = 11), and 6 (35.3%) of the 17 *BRCA1/2* variants were Ashkenazi Jewish founder mutations. Of all variants, 35 (40.7%) were of high penetrance, 24 (27.9%) were of moderate penetrance, 18 (20.9%) were of low or uncertain penetrance, and 10 (11.6%) were in a gene associated with an autosomal recessive cancer-associated syndrome. Sixty-five variants (75.6%) were in genes associated with DDR; of these, 12 were in the MMR-associated genes *MSH2* (n = 8), *MSH6* (n = 2), and *MLH1* (n = 2; Fig 2A).

Correlation Between Germline Genotype and Tumor Phenotype

Of 54 germline variants in DDR genes (excluding MMR genes), LOH/somatic mutation in the tumor was present in 18 patients (33.3%), including carriers of *ATM* (n = 2 of 3), *ERCC2* (n = 3 of 3), *BRCA2* (n = 6 of 9), and *BRCA1* (n = 2 of 8), among others (Fig 2B). In the identified cohort, all tumors from patients with a germline MMR variant had either MSI-H status or immunohistochemistry showing deficient MMR protein staining.

Estimate of UC Risk Associated With Observed Variants

To estimate population frequencies of germline P/LP variants in genes seen in the UC cohort, we analyzed allele frequencies of these genes in the ExAC dataset. *BRCA2* and *MSH2* showed statistically significant increased risk in UC patients compared with ExAC (odds ratio, 3.7; 95% CI, 1.5 to 7.8; $P < .003$ for *BRCA2*; and odds ratio, 4.6; 95% CI, 1.8 to 9.8; $P < .001$ for *MSH2*; Table 2).

Clinical Characteristics Associated With P/LP Variants

The only clinical characteristic available for analysis for patients in the anonymized cohort was site of tumor. In patients with bladder primaries from both the anonymized and identified cohorts, 39 (8.4%) had moderate- or high-risk P/LP variants. Conversely, in patients with UT primaries, 16 (14.0%) had moderate- or high-risk P/LP variants, including 10 (8.8%) with MMR mutations.

In the identified cohort, patients with P/LP variants were more likely to have early age of onset (age ≤ 45 years) compared with patients with no germline variant (22% *v* 6%; $P = 0.01$; Table 3). Six of 14 patients with early-onset UC had germline variants, including in *MSH2* (n = 2), *BRCA1* (n = 2), *MSH6* (n = 1), and *BRCA2* (n = 1). Presence of P/LP variants was not statistically significantly associated with a positive family history of UC, non-UC malignancy, smoking history, or metastasis at diagnosis, but presence was associated with Ashkenazi Jewish ancestry, reflective of founder mutations in *BRCA1*, *BRCA2*, and *CHEK2*.

Of 9 patients with MMR variants in the identified cohort, all but one had a UT tumor as the primary site of malignancy. The median age of diagnosis of UC was 58 years (range, 31-84 years), and UC was the first malignancy in 6 patients. Although none reported a family history of bladder or UT cancer, 4 reported relatives with cancers of unknown origin or kidney cancer, not otherwise specified.

Given the association of MSI-H status or deficient MMR tumors and response to immunotherapy in other malignancies, we explored response in the four patients in the identified cohort who had germline MMR variants (all MSI-H tumors) and who had received immunotherapy. All 4 had presented initially with metastatic disease and had received platinum chemotherapy as first-line treatment. Three experienced progression of disease after platinum chemotherapy followed by a complete response after immunotherapy (Appendix Fig A1, online only). The fourth patient experienced progression of disease on both platinum chemotherapy and immunotherapy.

Comparisons to Clinical Genetics Referral Criteria

In the identified cohort, detailed family history was obtained by interview for 29 (85.3%) of the patients with P/LP variants; for the remainder of patients, data were extracted from the electronic medical record. Only 9 of 27 patients with high- or moderate-penetrance variants had undergone clinical genetic testing or attended clinical genetic counseling before receiving genetic test results through the protocol. Of patients with high-penetrance P/LP variants, 6 patients (26.3%) would not have been referred for germline testing according to published guidelines (one each with *MSH2*, *MSH6*, *BRCA1*, *SDHA*, and *TP53*). The patient with a *TP53* variant, diagnostic of Li-Fraumeni syndrome, had a father with bladder cancer, but the patient did not meet criteria for any genetic testing. Of patients with

TABLE 1. Patient Characteristics

Characteristic	No. (%)		
	Identified Cohort (n = 169)	Anonymized Cohort (n = 417)	Total (N = 586)
Age, years			
Median (range)	63 (31-84)	64 (25-87)	63 (25-87)
≤ 45 years	14 (8.3)	28 (6.7)	42 (7.2)
≥ 46	155 (91.7)	388 (93.0)	543 (92.7)
Sex			
Female	41 (24.3)	111 (26.6)	152 (25.9)
Male	128 (75.7)	306 (73.4)	434 (74.1)
Race or ethnic background			
White	146 (86.4)	366 (87.8)	512 (87.4)
Hispanic	2 (1.2)	4 (1.0)	6 (1.0)
African American	5 (3.0)	13 (3.1)	18 (3.1)
Asian or Pacific Islander	5 (3.0)	12 (2.9)	17 (2.9)
Other or unknown	11 (6.5)	22 (5.3)	33 (5.6)
Ashkenazi Jewish ancestry			
Yes	31 (18.3)	76 (18.2)	107 (18.3)
No	97 (57.4)	220 (52.8)	317 (54.1)
Unknown	41 (24.3)	121 (29.0)	162 (27.7)
Other primary malignancy			
Yes	39 (23.1)	73 (17.5)	112 (19.1)
No	130 (76.9)	343 (82.3)	473 (80.7)
Unknown	0	1 (0.2)	1 (0.2)
Family history of urothelial cancer			
Yes	18 (10.7)	24 (5.8)	42 (7.2)
No	151 (89.3)	391 (93.8)	542 (92.5)
Unknown	0	2 (0.5)	2 (0.3)
Tobacco use history			
Ever	103 (60.9)	273 (65.5)	376 (64.2)
Never	66 (39.1)	142 (34.1)	208 (35.5)
Unknown	0	2 (0.5)	2 (0.3)
Site of primary malignancy			
Bladder/urethra	117 (69.2)	346 (83.0)	463 (79.0)
Renal pelvis/ureter	48 (28.4)	66 (15.8)	114 (19.5)
Both or unknown	4 (2.4)	5 (1.2)	9 (1.5)
Histologic subtype			
Urothelial carcinoma	169 (100.0)	393 (94.2)	562 (95.9)
Adenocarcinoma	0	16 (3.8)	16 (2.7)
Other	0	8 (1.9)	8 (1.4)
Stage at diagnosis			
Non–muscle invasive bladder	44 (26.0)	209 (50.1)	253 (43.2)
Muscle-invasive bladder	56 (33.1)	102 (24.5)	158 (27.0)
Localized upper tract	35 (20.7)	51 (12.2)	86 (14.7)
Metastatic	34 (20.1)	43 (10.3)	77 (13.1)
Unknown/other	0	12 (2.9)	12 (2.1)

(continued on following page)

TABLE 1. Patient Characteristics (continued)

Characteristic	No. (%)		
	Identified Cohort (n = 169)	Anonymized Cohort (n = 417)	Total (N = 586)
Stage at time of analysis			
Nonmetastatic	53 (31.4)	182 (43.7)	235 (40.1)
Metastatic	116 (68.6)	234 (56.1)	350 (59.7)
Unknown	0	1 (0.2)	1 (0.2)

NOTE. Cohort demographic and clinical characteristics are provided for the 586 patients who consented to tumor-normal testing. Patients in the identified cohort additionally consented to receive germline results.

moderate-penetrance variants, 7 (87.5%) would not have been referred (Appendix Table A5, online only).

DISCUSSION

This study shows that, in patients with UC, clinically significant P/LP germline variants, particularly in DDR genes, frequently are present. Multiple epidemiologic studies have identified an increased familial risk of UC. However, to date, the only identified hereditary cancer syndrome associated with increased UC risk is Lynch syndrome, which is caused by inactivating mutations in the MMR-associated genes *MSH2*, *MSH6*, *MLH1*, *PMS2*, and *EPCAM*.[3,28,29] Patients with Lynch syndrome have an up to 12% cumulative risk of urinary tract cancer; although the risk is greater for UT UC, there also may be an increased risk for bladder UC.[7,8,30,31] Studies estimating prevalence of Lynch syndrome in patients with UC, however, have been limited. In two studies looking at unselected patients with UT UC, 7% of tumors had deficient MMR protein expression or were MSI-H, usually a necessary but not sufficient biomarker for Lynch syndrome.[32,33] In our study, 2.1% overall and 8.7% of patients with UT tumors had Lynch syndrome, and, for those in the identified cohort, 6 of 9 had UC as the first malignancy. Immunohistochemistry analysis of tumors for loss of MMR protein expression or tumor MSI analysis is standard practice for colorectal and endometrial cancers, for which the incidence of Lynch syndrome ranges from 2% to 5%.[34] A recent pan-cancer study showed that, among patients with MSI-high/intermediate UC, 37.5% had a germline MMR variant diagnostic of Lynch syndrome, the highest prevalence of all cancer types analyzed.[35] The increased incidence of Lynch syndrome in our cohort, and the high prevalence of Lynch syndrome in those with MSI-high/intermediate tumors, supports consideration of germline or tumor screening for those with UT UC.

In our cohort, 9% of patients had a germline DDR mutation in a gene other than those in MMR. Dysregulation of DNA repair is implicated in the carcinogenesis of UC. DDR somatic mutations are frequent in UC.[25,36] For example, the nucleotide excision repair pathway gene *ERCC2* was somatically mutated in 9% of UC in TCGA, and common polymorphisms in *ERCC2*, *NBN*, and *XPC* are associated

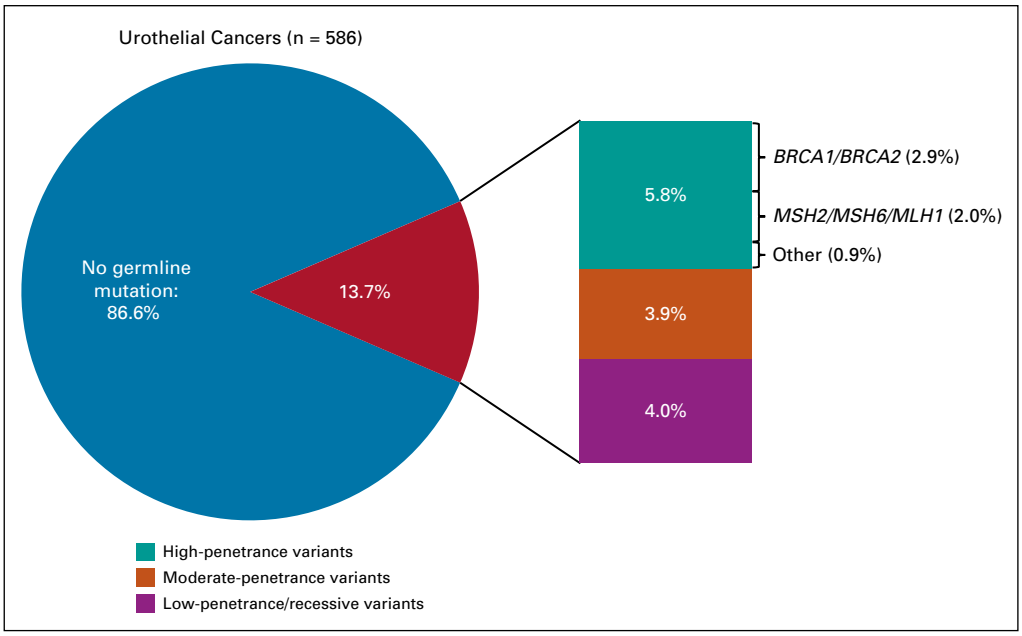

FIG 1. Frequency and penetrance of germline variants in patients with urothelial cancer. Frequency of pathogenic/likely pathogenic (P/LP) germline variants identified in 586 patients with UC. Pie chart shows that 13.7% (n = 80 patients) had a germline P/LP variant; 9.7% (n = 57) had high- or moderate-penetrance variants.

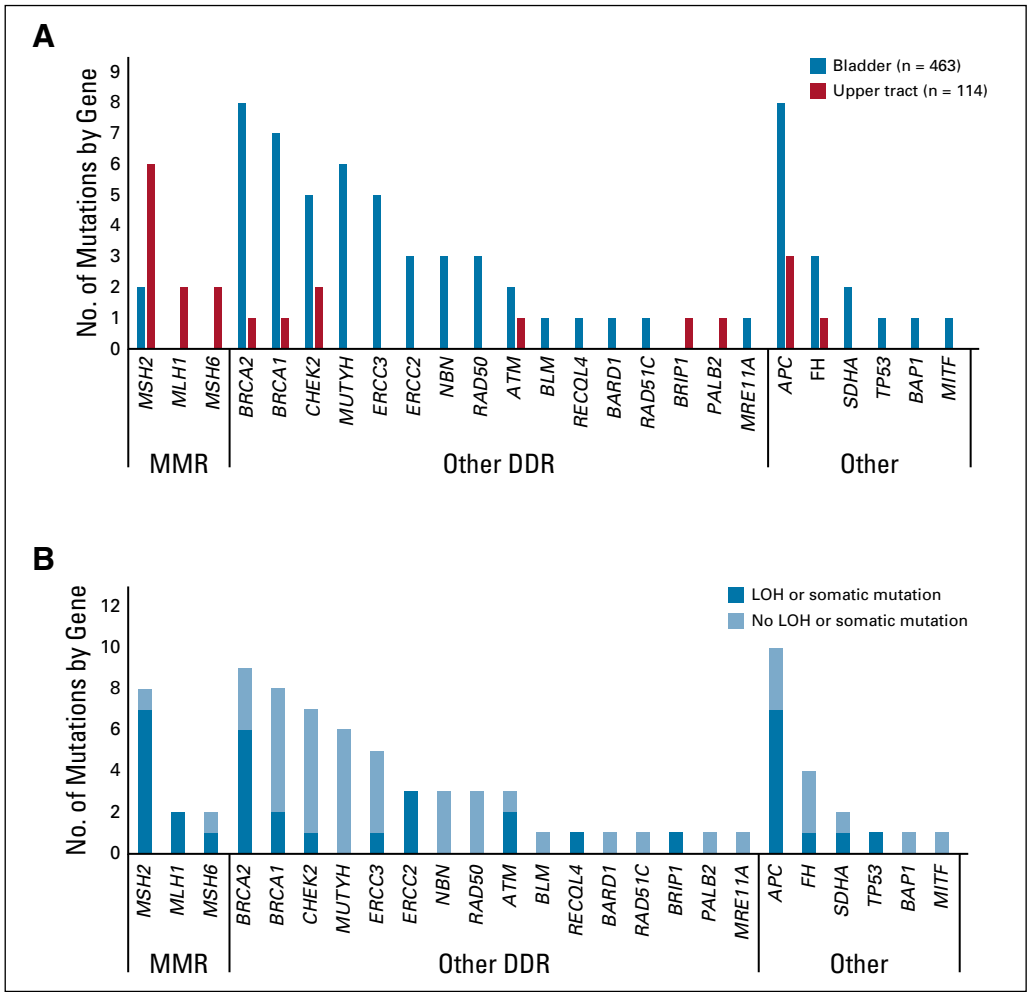

FIG 2. Germline pathogenic/likely pathogenic variants by site of tumor origin and tumors with loss of heterozygosity (LOH) or second somatic mutation in the tumor in 586 patients. (A) Number of mutations in each gene by tumor type and (B) in germline-positive occurrences with LOH or second somatic mutation in same locus. DDR, DNA-damage repair; MMR, mismatch repair.

with bladder cancer risk.[13,37,38] To further investigate the pathogenic role of DDR genes, we compared allele frequencies of the most frequently mutated genes in UC with frequencies in individuals in ExAC without cancer, and we found significantly increased frequencies of mutations in *BRCA2* and *MSH2* in UC. We also found LOH in 6 of 9 tumors in patients with germline variants in *BRCA2* and 3 of 3 tumors in patients with germline *ERCC2* variants. Of note, the current number of carriers is too small to analyze enrichment for second allele loss in tumors for germline

TABLE 2. Comparison of Allele Frequencies in All Patients Versus ExAC

Gene	UC Occurrence Allele Count	UC Occurrence Allele Number	Allele Count ExAC	Allele ExAC	Odds Ratio (95% CI)	P
ATM	3	1,172	183	106,194	1.49 (0.15 to 5.7)	.46
BRCA1	4	1,164	85	106,206	4.29 (0.67 to 14.6)	.02
BRCA2	7	1,168	173	106,187	3.68 (1.01 to 9.5)	.004*
MSH2	7	1,172	133	102,035	4.58 (1.26 to 11.9)	.001*
NBN	3	1,172	65	106,189	4.18 (0.43 to 16.7)	.04
RAD50	3	1,172	392	106,179	0.69 (0.07 to 2.6)	.81

NOTE. Odds ratio and CIs calculated with Bonferroni correction. Only genes with three or more occurrences in the UC cohort were considered. Ashkenazi Jewish *BRCA1/2* founder mutations were excluded.

Abbreviation: UC, urothelial carcinoma.

*Statistically significant with Bonferroni-corrected α = .05/6 = .0083.

TABLE 3. Clinical Characteristics by Moderate-/High-Penetrance Variants in Identified Cohort

Characteristic	Any Moderate/High Penetrance (n = 27)	No Moderate/High Penetrance (n = 142)	P
Age, years			
≤ 45	6 (22)	8 (6)	.01
≥ 46	21 (78)	134 (94)	
Sex			
Male	19 (70)	109 (77)	.47
Female	8 (30)	33 (23)	
Ashkenazi Jewish ancestry			
Yes	11 (41)	20 (14)	.005
No	13 (48)	84 (59)	
Unknown	3 (11)	38 (27)	
Tobacco use history			
Current or former	15 (56)	88 (62)	.53
Never	12 (44)	54 (38)	
Family history of UC			
Yes	2 (7)	17 (12)	.74
No	25 (93)	125 (88)	
History of second malignancy			
Yes	7 (26)	32 (23)	.80
No	20 (74)	110 (77)	
Site of primary malignancy			
Bladder/urethra	16 (59)	101 (72)	.32
Renal pelvis/ureter	11 (41)	37 (26)	
Both/unknown	0	2 (1)	
Stage at diagnosis			
Nonmetastatic	24 (89)	111 (78)	.30
Metastatic	3 (11)	31 (22)	
Stage at last follow-up*			
Nonmetastatic	12 (44)	41 (29)	.12
Metastatic	15 (56)	101 (71)	

Abbreviation: UC, urothelial carcinoma.
*Median length of follow-up was 4.1 years (range, 1.5-23.2 years).

events compared with the background loss of first somatic allele in these genes. Pathogenic nucleotide excision repair germline mutations have not been described previously in UC, although heterozygous germline mutations in *ERCC2* and *ERCC3* have been associated with increased risk of sarcoma and breast cancer.[38,39] Additional studies will be needed to determine the role of these germline mutations in the pathogenicity of UC.

Germline MMR and other DDR mutations have potential implications for treatment selection. Somatic mutations in *ERCC2*, *ATM*, *RB1*, *FANCC*, and other DDR genes are correlated with improved responses to platinum-based chemotherapy in patients with UC.[12,13,40,41] Whether germline pathogenic mutations in these genes also are associated with improved outcomes must be explored. Preclinical models with *ERCC2* and *ERCC3* heterozygous knockouts show a hypomorphic functionality when exposed to DNA damaging agents, supporting a plausible mechanism for sensitivity to platinum therapies.[23,25] Although tumor MMR deficiency predicts response to PD-1 blockade in other solid tumors, to date, no large studies correlating MMR deficiency and response to PD-1 or PD-L1 blockade have included patients with UC.[11] In this study, all patients with germline MMR mutations had MSI-H or MMR-deficient tumors, and 3 of 4 patients with Lynch syndrome had complete responses to immunotherapy after they experienced progression on chemotherapy.[11,42] Our findings suggest that germline DDR alterations should be included with somatic alterations when assessing for correlations between therapeutic benefit.

Finally, a quarter of patients with high-penetrance germline variants would not have been detected by guidelines-directed testing. Identification of germline mutations in these patients may allow for enhanced screening and early detection of hereditary cancers in those families for whom testing would not have been undertaken. Several individuals became the index cases (first detected cancer) in their families, which then led to cascade testing of other family members. Interestingly, among those with a family history of UC or personal history of other cancers, there was no increased incidence of P/LP germline variants. This may be explained in part by incomplete information available to patients—for example, of 8 patients with Lynch syndrome, none reported a family history of bladder or UT cancer, but 4 did report relatives with possible kidney cancer or of unclear origin, which may reflect UT cancers. Analysis in a larger cohort also may reveal whether personal history of cancer is associated with presence of germline alterations.

This study had several limitations. It was retrospective in nature and had a limited sample size; its findings will require validation in other cohorts. Given the smaller number of identified patients with clinically annotated data, associations between clinical features and prevalence of mutations may be limited by numbers. The study was conducted at a comprehensive cancer center with regional referral patterns, so the patient population may differ from that in the general community. For example, the median age of diagnosis in the cohort was 63 years, compared with approximately 70 years in the United States, and there was under-representation of nonwhite patients.[43] We did observe a lower prevalence of germline mutations in the anonymized cohort, in which germline results were not returned. Although physicians were not instructed to select patients according to suspicion of an inherited syndrome, individuals consenting to return of germline results may

have be more motivated to do so because of family history. We performed targeted exome sequencing of known cancer-predisposition genes; agnostic whole-exome sequencing could yield associations among novel genes or variants and risk of UC. LOH analysis was exploratory and was not corrected for possible increased background LOH in those genes, which also merits more study in larger cohorts.

This study demonstrates that germline mutations in patients with UC occur predominantly in DDR genes, with increased frequency of *BRCA2* and *MSH2*. Traditional criteria to identify those at risk for hereditary syndromes only identified a fraction of patients. There is potential value of expanded germline analysis in UC, particularly in patients of young age at diagnosis and those with UT tumors. The genes found to be mutated were associated with increased risk for cancers other than UC; thus, their identification likely will have substantial implications for directed cancer screening in patients and their families.

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EQUAL CONTRIBUTION

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PRIOR PRESENTATION

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Cancer Susceptibility Mutations in Patients With Urothelial Malignancies

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APPENDIX

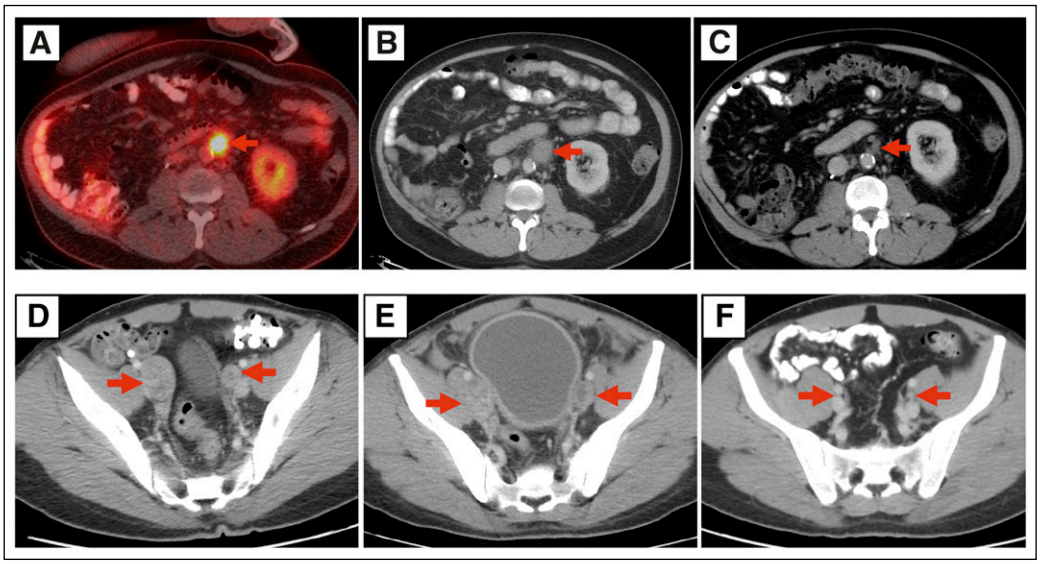

FIG A1. Response to chemotherapy and immunotherapy in two patients with Lynch syndrome. (A) 18-Fluorodeoxyglucose positron emission tomography/computer tomography (PET/CT) image reveals a left para-aortic lymph node measuring 1.9 cm in largest dimension (red arrow) before initiation of paclitaxel. (B) Post-treatment CT after six infusions of paclitaxel shows enlargement of lymph node to 2.4 cm. (C) Post-treatment CT after six infusions of atezolizumab shows reduction in lymph node size to 0.9 cm, considered complete response by RECIST v 1.1 criteria. (D) CT image in another patient reveals right and left external iliac lymph nodes measuring 2.8 cm and 2.3 cm in largest dimensions, respectively (red arrows), before initiation of platinum-based chemotherapy. (E) Post-treatment CT after three cycles of platinum-based therapy shows right lymph node now to 2.7 cm and left to 3.2 cm. Treatment with chemotherapy was stopped, and the patient proceeded to treatment with a programmed death ligand 1 (PDL-1) inhibitor. (F) Post-treatment CT after 24 infusions of PDL-1 inhibitor shows disappearance of right lymph node and reduction of left lymph node to 0.6 cm. After 26 months on immune therapy, the patient proceeded to radical cystectomy with lymph node resection. Pathology review showed a complete pathologic response without residual carcinoma.

TABLE A1. Genes on MSK-IMPACT and Syndromes Associated With Germline Mutations

Gene	Syndrome
ABL1	
ACVR1	
AKT1	
AKT2	
AKT3	
ALK	Familial neuroblastoma
ALOX12B	
AMER1	
ANKRD11	
APC	Familial adenomatous polyposis
AR	
ARAF	
ARID1A	
ARID1B	
ARID2	
ARID5B	
ASXL1	
ASXL2	
ATM	Ataxia-telangiectasia; *ATM*-related cancer risk
ATR	
ATRX	
AURKA	
AURKB	
AXIN1	
AXIN2	
AXL	
B2M	
BABAM1	
BAP1	Mesothelioma, uveal melanoma, RCC
BARD1	Hereditary breast and ovarian cancer syndrome
BBC3	
BCL10	
BCL2	
BCL2L11	
BCL2L1	
BCL6	
BCOR	
BIRC3	
BLM	Bloom syndrome
BMPR1A	Juvenile polyposis syndrome
BRAF	
BRCA1	Hereditary breast and ovarian cancer syndrome

(continued in next column)

TABLE A1. Genes on MSK-IMPACT and Syndromes Associated With Germline Mutations (continued)

Gene	Syndrome
BRCA2	Hereditary breast and ovarian cancer syndrome; Fanconi anemia
BRD4	
BRIP1	*BRIP1*-related cancer; Fanconi anemia
BTK	
KNSTRN	
CALR	
CARD11	
CARM1	
CASP8	
CBFB	
CBL	
CCND1	
CCND2	
CCND3	
CCNE1	
CD274	
CD276	
CD79A	
CD79B	
CDC42	
CDC73	
CDH1	Hereditary diffuse gastric cancer
CDK12	
CDK4	Familial cutaneous melanoma
CDK6	
CDK8	
CDKN1A	
CDKN1B	
CDKN2A	Familial cutaneous melanoma
CDKN2B	
CDKN2C	
CEBPA	
CENPA	
CHEK1	
CHEK2	*CHEK2*-related cancer
CIC	
CREBBP	
CRKL	
CRLF2	
CSDE1	
CSF1R	
CSF3R	

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TABLE A1. Genes on MSK-IMPACT and Syndromes Associated With Germline Mutations (continued)

Gene	Syndrome
CTCF	
CTLA4	
CTNNB1	
CUL3	
CXCR4	
CYLD	
CYSLTR2	
DAXX	
DCUN1D1	
DDR2	
DICER1	*DICER1*-related disorders
DIS3	
DNAJB1	
DNMT1	
DNMT3A	
DNMT3B	
DOT1L	
DROSHA	
DUSP4	
E2F3	
EED	
EGFL7	
EGFR	Familial lung cancer
EIF1AX	
AGO2	
EIF4A2	
EIF4E	
ELF3	
EP300	
EPAS1	
EPCAM	Lynch syndrome
EPHA3	
EPHA5	
EPHA7	
EPHB1	
ERBB2	
ERBB3	
ERBB4	
ERCC2	Xeroderma pigmentosum
ERCC3	Xeroderma pigmentosum/ Hereditary breast cancer syndrome
ERCC4	
ERCC5	

(continued in next column)

TABLE A1. Genes on MSK-IMPACT and Syndromes Associated With Germline Mutations (continued)

Gene	Syndrome
ERF	
ERG	
ERRFI1	
ESR1	
ETV1	
ETV6	
EZH1	
EZH2	
FAM175A	Hereditary breast cancer syndrome
FAM46C	
FAM58A	
FANCA	
FANCC	
FAT1	
FBXW7	
FGF19	
FGF3	
FGF4	
FGFR1	
FGFR2	
FGFR3	
FGFR4	
FH	Hereditary leiomyomatosis and renal cell cancer
FLCN	Birt-Hogg-Dubé syndrome
FLT1	
FLT3	
FLT4	
FOXA1	
FOXL2	
FOXO1	
FOXP1	
FUBP1	
FYN	
GATA1	
GATA2	Familial MDS-AML
GATA3	
GLI1	
GNA11	
GNAQ	
GNAS	
GPS2	
GRIN2A	
GSK3B	

(continued on following page)

TABLE A1. Genes on MSK-IMPACT and Syndromes Associated With Germline Mutations (continued)

Gene	Syndrome
H3F3A	
H3F3B	
H3F3C	
HGF	
HIST1H1C	
HIST1H2BD	
HIST1H3A	
HIST1H3B	
HIST1H3C	
HIST1H3D	
HIST1H3E	
HIST1H3F	
HIST1H3G	
HIST1H3H	
HIST1H3I	
HIST1H3J	
HIST2H3C	
HIST2H3D	
HIST3H3	
HLA-A	
HLA-B	
HNF1A	
HOXB13	
HRAS	Costello syndrome
ICOSLG	
ID3	
IDH1	
IDH2	
IFNGR1	
IGF1	
IGF1R	
IGF2	
IKBKE	
IKZF1	
IL10	
IL7R	
INHA	
INHBA	
INPP4A	
INPP4B	
INPPL1	
INSR	
IRF4	

(continued in next column)

TABLE A1. Genes on MSK-IMPACT and Syndromes Associated With Germline Mutations (continued)

Gene	Syndrome
IRS1	
IRS2	
JAK1	
JAK2	Familial thrombocytosis
JAK3	
JUN	
KDM5A	
KDM5C	
KDM6A	
KDR	
KEAP1	
KIT	Hereditary gastrointestinal stromal tumors
KLF4	
KRAS	Noonan syndrome
LATS1	
LATS2	
LMO1	
LYN	
MALT1	
MAP2K1	
MAP2K2	
MAP2K4	
MAP3K13	
MAP3K14	
MAP3K1	
MAPK1	
MAPK3	
MAPKAP1	
MAX	Hereditary paraganglioma-pheochromocytoma syndromes
MCL1	
MDC1	
MDM2	
MDM4	
MED12	
MEF2B	
MEN1	Multiple endocrine neoplasia, type 1
MET	Hereditary papillary renal carcinoma
MGA	
MITF	Familial melanoma and renal cell carcinoma
MLH1	Lynch syndrome
MLL2	
MLL3	

(continued on following page)

TABLE A1. Genes on MSK-IMPACT and Syndromes Associated With Germline Mutations (continued)

Gene	Syndrome
MLL4	
MLL	
MPL	
MRE11A	Ataxia-telangiectasia-like disorder (recessive); breast cancer
MSH2	Lynch syndrome
MSH3	
MSH6	Lynch syndrome
MSI1	
MSI2	
MST1	
MST1R	
MTOR	
MUTYH	*MUTYH*-associated polyposis
MYC	
MYCL1	
MYCN	
MYD88	
MYOD1	
NBN	Nijmegen breakage syndrome; *NBN*-related cancer risk
NCOA3	
NCOR1	
NEGR1	
NF1	Neurofibromatosis, type 1
NF2	Neurofibromatosis, type 2
NFE2L2	
NFKBIA	
NKX2-1	
NKX3-1	
NOTCH1	
NOTCH2	
NOTCH3	
NOTCH4	
NPM1	
NRAS	Autoimmune lymphoproliferative syndrome
NSD1	
NTHL1	
NTRK1	
NTRK2	
NTRK3	
NUF2	
NUP93	

(continued in next column)

TABLE A1. Genes on MSK-IMPACT and Syndromes Associated With Germline Mutations (continued)

Gene	Syndrome
PAK1	
PAK7	
PALB2	*PALB2*-related cancer; Fanconi anemia
PARK2	
PARP1	
PAX5	B cell precursor acute lymphoblastic leukemia
PBRM1	
PDCD1	
PDCD1LG2	
PDGFRA	Hereditary gastrointestinal stromal tumors
PDGFRB	
PDPK1	
PGR	
PHOX2B	Familial neuroblastoma; congenital central hypoventilation syndrome
PIK3C2G	
PIK3C3	
PIK3CA	
PIK3CB	
PIK3CD	
PIK3CG	
PIK3R1	
PIK3R2	
PIK3R3	
PIM1	
PLCG2	
PLK2	
PMAIP1	
PMS1	
PMS2	Lynch syndrome
PNRC1	
POLD1	
POLE	Colorectal cancer and endometrial cancer
PPARG	
PPM1D	
PPP2R1A	
PPP4R2	
PPP6C	
PRDM14	
PRDM1	
PREX2	
PRKAR1A	
PRKCI	

(continued on following page)

TABLE A1. Genes on MSK-IMPACT and Syndromes Associated With Germline Mutations (continued)

Gene	Syndrome
PRKD1	
PTCH1	Nevoid basal cell carcinoma syndrome
PTEN	*PTEN* hamartoma tumor syndrome
PTP4A1	
PTPN11	
PTPRD	
PTPRS	
PTPRT	
RAB35	
RAC1	
RAC2	
RAD21	
RAD50	Nijmegen breakage syndrome-like disorder
RAD51	Hereditary breast cancer
RAD51B	Hereditary breast cancer
RAD51C	*RAD51C*-related cancer; Fanconi anemia
RAD51D	Hereditary ovarian cancer
RAD52	
RAD54L	
RAF1	
RARA	
RASA1	
RB1	Retinoblastoma
RBM10	
RECQL4	Rothmund-Thomson syndrome
RECQL	
REL	
RET	Multiple endocrine neoplasia, type 2
RFWD2	
RHEB	
RHOA	
RICTOR	
RIT1	
RNF43	
ROS1	
RPS6KA4	
RPS6KB2	
RPTOR	
RRAGC	
RRAS2	
RRAS	
RTEL1	

(continued in next column)

TABLE A1. Genes on MSK-IMPACT and Syndromes Associated With Germline Mutations (continued)

Gene	Syndrome
RUNX1	Familial platelet disorder with predisposition to acute myelogenous leukemia
RXRA	
RYBP	
SDHA	Hereditary paraganglioma-pheochromocytoma syndromes
SDHAF2	Hereditary paraganglioma-pheochromocytoma syndromes
SDHB	Hereditary paraganglioma-pheochromocytoma syndromes
SDHC	Hereditary paraganglioma-pheochromocytoma syndromes
SDHD	Hereditary paraganglioma-pheochromocytoma (PGL/PCC) syndromes
SESN1	
SESN2	
SESN3	
SETD2	
SETD8	
SF3B1	
SH2B3	
SH2D1A	
SHOC2	
SHQ1	
SLX4	
SMAD2	
SMAD3	Thoracic aortic aneurysms and aortic dissections
SMAD4	Juvenile polyposis syndrome
SMARCA4	Rhabdoid tumor predisposition syndrome type 2
SMARCB1	Rhabdoid tumor predisposition syndrome type 1
SMARCD1	
SMO	
SMYD3	
SOCS1	
SOS1	
SOX17	
SOX2	
SOX9	
SPEN	
SPOP	
SPRED1	
SRC	
SRSF2	
STAG2	

(continued on following page)

TABLE A1. Genes on MSK-IMPACT and Syndromes Associated With Germline Mutations (continued)

Gene	Syndrome
STAT3	
STAT5A	
STAT5B	
STK11	Peutz-Jeghers syndrome
STK19	
STK40	
SUFU	Medulloblastoma
SUZ12	
SYK	
TAP1	
TAP2	
TBX3	
TCEB1	
TCF3	
TCF7L2	
TEK	
TERT	Familial pulmonary fibrosis; dyskeratosis congenita
TET1	
TET2	
TGFBR1	Thoracic aortic aneurysms and aortic dissections
TGFBR2	Thoracic aortic aneurysms and aortic dissections
TMEM127	Familial pheochromocytoma syndrome
TMPRSS2	
TNFAIP3	
TNFRSF14	
TOP1	
TP53	Li-Fraumeni syndrome
TP53BP1	
TP63	
TRAF2	
TRAF7	
TSC1	Tuberous sclerosis complex
TSC2	Tuberous sclerosis complex
TSHR	
U2AF1	
UPF1	
VEGFA	
VHL	Von Hippel-Lindau syndrome; familial erythrocytosis, type 2
VTCN1	
WHSC1	
WHSC1L1	

(continued in next column)

TABLE A1. Genes on MSK-IMPACT and Syndromes Associated With Germline Mutations (continued)

Gene	Syndrome
WT1	Wilms tumor-aniridia-genital anomalies-retardation syndrome, Denys-Drash syndrome, Frasier syndrome, and isolated Wilms tumor
WWTR1	
XIAP	
XPO1	
XRCC2	
YAP1	
YES1	
ZFHX3	
ZRSR2	

NOTE. Genes in bold were included for germline analysis.

Abbreviations: MDS-AML, myelodysplastic syndrome/acute myeloid leukemia; RCC, renal cell carcinoma.

TABLE A2. DDR Gene Panel

DDR Pathway					
MMR	**NER**	**HR**	**FA**	**Checkpoint**	**Other**
MLH1	*ERCC2*	*BRCA1*	*BRCA2*	*ATM*	*POLE*
MSH2	*ERCC3*	*MRE11A*	*BRIP1*	*ATR*	*MUTYH*
MSH6	*ERCC4*	*NBN*	*FANCA*	*CHEK1*	*PARP1*
PMS1	*ERCC5*	*RAD50*	*FANCC*	*CHEK2*	*RECQL4*
PMS2	—	*RAD51*	*PALB2*	*MDC1*	—
—	—	*RAD51B*	*RAD51C*	—	—
—	—	*RAD51D*	*BLM*	—	—
—	—	*RAD52*	—	—	—
—	—	*RAD54L*	—	—	—

Abbreviations: DDR, DNA-damage repair; HR, homologous recombination; FA, Fanconi anemia; MMR, mismatch repair; NER, nucleotide excision repair.

TABLE A3. Ashkenazi Jewish and European Founder Mutations

BRCA1 c.68_69delAG (p.Glu23Valfs*17)
BRCA1 c.5266dupC (p.Gln1756Profs*74)
BRCA2 c.5946delT (p.Ser1982Argfs*22)
CHEK2 c.1100delC (p.Thr367Metfs*15)
CHEK2 c.1283C>T (p.Ser428Phe)
APC c.3920T>A (p.Ile1307Lys)
MUTYH c.1187G>A (p.Gly396Asp)
MUTYH c. 536A>G (p.Tyr179Cys)
ERCC3 c.325C>T (p.Arg109X)

TABLE A4. Detail on Pathogenic/Likely Pathogenic Germline Variants

Study ID	Gene	Variant	Protein	Penetrance	Zygosity	Site	DDR Gene	Cohort
A1	*APC*	c.3920T>A	p.Ile1307Lys	Low	No LOH	Bladder	No	Anonymized
A2	*APC*	c.3920T>A	p.Ile1307Lys	Low	LOH	Bladder	No	Anonymized
A3	*APC*	c.3920T>A	p.Ile1307Lys	Low	LOH	Bladder	No	Anonymized
A4	*APC*	c.3920T>A	p.Ile1307Lys	Low	LOH	Bladder	No	Anonymized
A5	*APC*	c.3920T>A	p.Ile1307Lys	Low	No LOH	Bladder	No	Anonymized
B1	*APC*	c.3920T>A	p.Ile1307Lys	Low	LOH	Bladder	No	Identified
B164	*APC*	c.3920T>A	p.Ile1307Lys	Low	LOH	Bladder	No	Identified
B23	*APC*	c.3920T>A	p.Ile1307Lys	Low	LOH	Bladder	No	Identified
A7	*ATM*	c.5932G>T	p.Glu1978Ter	Moderate	No LOH	Bladder	Yes	Anonymized
A8	*ATM*	c.7638_7646delTAGAATTTC	p.Arg2547_Ser2549del	Moderate	LOH	Bladder	Yes	Anonymized
A9	*BAP1*	c.1203T>G	p.Tyr401Ter	High	No LOH	Bladder	No	Anonymized
A10	*BARD1*	c.1652C>G	p.Ser551Ter	Moderate	No LOH	Bladder	Yes	Anonymized
A6	*APC*	c.3920T>A	p.Ile1307Lys	Low	No LOH	Upper tract	No	Anonymized
A11	*BRCA1*	c.5319dupC	p.Asn1774GlnfsTer56	High	No LOH	Bladder	Yes	Anonymized
A21	*CHEK2*	c.444+1G>A		Moderate	No LOH	Upper tract	Yes	Anonymized
A12	*BRCA1*	c.116G>A	p.Cys39Tyr	High	No LOH	Bladder	Yes	Anonymized
A13	*BRCA1*	c.68_69delAG	p.Glu23ValfsTer17	High	No LOH	Bladder	Yes	Anonymized
B13	*BRCA1*	c.1687C>T	p.Gln563*	High	No LOH	Bladder	Yes	Identified
B148	*BRCA1*	c.68_69delAG	p.Glu23Valfs*17	High	LOH	Bladder	Yes	Identified
B21	*BRCA1*	c.68_69delAG	p.Glu23Valfs*17	High	No LOH	Bladder	Yes	Identified
B22	*BRCA1*	c.5074G>C	p.ASp1692His	High	No LOH	Bladder	Yes	Identified
A14	*BRCA2*	c.5799_5802delCCAA	p.Asn1933LysfsTer29	High	No LOH	Bladder	Yes	Anonymized
A15	*BRCA2*	c.8537_8538delAG	p.Glu2846GlyfsTer22	High	LOH	Bladder	Yes	Anonymized
A16	*BRCA2*	c.1238delT	p.Leu413HisfsTer17	High	LOH	Bladder	Yes	Anonymized
A17	*BRCA2*	c.8869C>T	p.Gln2957Ter	High	No LOH	Bladder	Yes	Anonymized
A18	*BRCA2*	c.7878G>C	p.Trp2626Cys	High	No LOH	Bladder	Yes	Anonymized
A32	*MSH2*	c.1906G>C	p.Ala636Pro	High	Somatic	Upper tract	Yes	Anonymized
B23	*BRCA2*	c.5946delT	p.Ser1982Argfs*22	High	LOH	Bladder	Yes	Identified
B24	*BRCA2*	c.1796_1800delCTTAT	p.Ser599*	High	LOH	Bladder	Yes	Identified
B28	*BRCA2*	c.5946delT	p.Ser1982Argfs*22	High	LOH	Bladder	Yes	Identified
A19	*CHEK2*	c.1100delC	p.Thr367MetfsTer15	Moderate	No LOH	Bladder	Yes	Anonymized
A20	*CHEK2*	c.1283C>T	p.Ser428Phe	Moderate	No LOH	Bladder	Yes	Anonymized
B21	*CHEK2*	c.1283C>T	p.Ser428Phe	Moderate	No LOH	Bladder	Yes	Identified
B51	*CHEK2*	c.444+1G>A		Moderate	No LOH	Bladder	Yes	Identified
B57	*CHEK2*	c.1283C>T	p.Ser428Phe	Moderate	No LOH	Bladder	Yes	Identified
A40	*PALB2*	c.940C>T	p.Gln314Ter	High	No LOH	Upper tract	Yes	Anonymized
B109	*APC*	c.3920T>A	p.Ile1307Lys	Low	No LOH	Upper tract	No	Identified
B180	*BLM*	c.2207_2212delinsTAGATTC	p.Tyr736LeufsTer5	Recessive	No LOH	Bladder	Yes	Identified
B47	*CHEK2*	c.470T>C	p.Ile157Thr	Uncertain	LOH	Upper tract	Yes	Identified
A11	*ERCC2*	c.1847G>C	p.Arg616Pro	Recessive	LOH	Bladder	Yes	Anonymized
A25	*ERCC3*	c.325C>T	p.Arg109Ter	Moderate	No LOH	Bladder	Yes	Anonymized
A26	*ERCC3*	c.325C>T	p.Arg109Ter	Moderate	No LOH	Bladder	Yes	Anonymized
A22	*ERCC2*	c.2150C>G	p.Ala717Gly	Recessive	Somatic	Bladder	Yes	Anonymized

(continued on following page)

TABLE A4. Detail on Pathogenic/Likely Pathogenic Germline Variants (continued)

Study ID	Gene	Variant	Protein	Penetrance	Zygosity	Site	DDR Gene	Cohort
A6	*MSH2*	c.1906G>C	p.Ala636Pro	High	Somatic	Upper tract	Yes	Anonymized
A27	*ERCC3*	c.325C>T	p.Arg109Ter	Moderate	No LOH	Bladder	Yes	Anonymized
B20	*ERCC3*	c.325C>T	p.Arg109Ter	Moderate	No LOH	Bladder	Yes	Identified
A23	*ERCC2*	c.1847G>C	p.Arg616Pro	Recessive	LOH	Bladder	Yes	Anonymized
A24	*ERCC3*	c.576_583delCGTGATCC	p.Val193ArgfsTer8	Recessive	LOH	Bladder	Yes	Anonymized
A28	*FH*	c.1431_1433dupAAA	p.Lys477dup	Recessive	No LOH	Bladder	No	Anonymized
B101	*MSH2*	c.1906G>C	p.Ala636Pro	High	Somatic	Upper tract	Yes	Identified
A29	*FH*	c.1431_1433dupAAA	p.Lys477dup	Recessive	LOH	Bladder	No	Anonymized
B109	*MSH6*	c.3261dupC	p.Phe1088Leufs*5	High	No LOH	Upper tract	Yes	Identified
B111	*MSH6*	c.3463C>T	p.Gln1155*	High	Somatic	Upper tract	Yes	Identified
B161	*MITF*	c.952G>A	p.Glu318Lys	Moderate	No LOH	Bladder	No	Identified
A30	*MRE11A*	c.1222dupA	p.Thr408AsnfsTer49	Moderate	No LOH	Bladder	Yes	Anonymized
A31	*MSH2*	c.1255C>T	p.Gln419Ter	High	Somatic	Bladder	Yes	Anonymized
B100	*MSH2*	c.1216C>T	p.R406*	High	LOH	Bladder	Yes	Identified
B70	*FH*	c.1431_1433dupAAA	p.Lys477dup	Recessive	No LOH	Bladder	No	Identified
B14	*BRCA1*	c.68_69delAG	p.Glu23Valfs*17	High	CNLOH	Upper tract	Yes	Identified
B61	*FH*	c.1431_1433dupAAA	p.Lys477dup	Recessive	No LOH	Upper tract	No	Identified
A33	*MUTYH*	c. 536A>G	p.Tyr179Cys	Low	No LOH	Bladder	Yes	Anonymized
A34	*MUTYH*	c.1187G>A	p.Gly396Asp	Low	No LOH	Bladder	Yes	Anonymized
A35	*MUTYH*	c. 536A>G	p.Tyr179Cys	Low	No LOH	Bladder	Yes	Anonymized
B174	*MLH1*	c.1591delG	p.Val531Trpfs*4	High	LOH	Upper tract	Yes	Identified
A36	*MUTYH*	c.1187G>A	p.Gly396Asp	Low	No LOH	Bladder	Yes	Anonymized
A38	*NBN*	c.2140C>T	p.Arg714Ter	Moderate	No LOH	Bladder	Yes	Anonymized
A39	*NBN*	c.657_661delACAAA	p.Lys219AsnfsTer16	Moderate	No LOH	Bladder	Yes	Anonymized
B119	*NBN*	c.2T>C	p.Met1Thr	Moderate	No LOH	Bladder	Yes	Identified
A41	*RAD50*	c.326_329delCAGA	p.Thr109AsnfsTer20	Moderate	No LOH	Bladder	Yes	Anonymized
A42	*RAD50*	c.1270_1271delCT	p.Leu424GlufsTer7	Moderate	No LOH	Bladder	Yes	Anonymized
A43	*RAD50*	c.2467C>T	p.Arg823Ter	Moderate	No LOH	Bladder	Yes	Anonymized
B139	*RAD51C*	Exons 1-3 deletion		Moderate	No LOH	Bladder	Yes	Identified
A37	*MUTYH*	c.1187G>A	p.Gly396Asp	Low	No LOH	Bladder	Yes	Anonymized
B43	*BRIP1*	c.918+1G>A		Moderate	LOH	Upper tract	Yes	Identified
B118	*MUTYH*	c.536A>G	p.Tyr179Cys	Low	No LOH	Bladder	Yes	Identified
B5	*ATM*	c.748C>T	p.Arg250*	Moderate	CNLOH	Upper tract	Yes	Identified
A45	*SDHA*	c.245_252delAGGCAGGG	p.Glu82ValfsTer2	High	LOH	Bladder	No	Anonymized
B159	*SDHA*	c.1A>G	p.Met1Val	High	No LOH	Bladder	No	Identified
A44	*RECQL4*	c.2464-1G>C		Recessive	LOH	Bladder	Yes	Anonymized
B127	*TP53*	c.374C>T	p.Thr125Met	High	Somatic	Bladder	No	Identified
B71	*MLH1*	c.790+2T>C		High	LOH	Upper tract	Yes	Identified
B73	*MSH2*	c.1046C>G	p.Pro349Arg	High	Somatic	Upper tract	Yes	Identified
B79	*MSH2*	c.942+3A>T		High	Hom del	Upper tract	Yes	Identified
B85	*MSH2*	c.1784T>G	p.L595R	High	No LOH	Upper tract	Yes	Identified
A46	*APC*	c.3920T>A	p.Ile1307Lys	Low	LOH	Upper tract	No	Anonymized
A46	*BRCA2*	c.5722_5723delCT	p.Leu1908ArgfsTer2	High	LOH	Upper tract	Yes	

Abbreviations: CNLOH, copy neutral loss of heterozygosity; DDR, DNA-damage repair; Hom del, homologous deletion; LOH, loss of heterozygosity; mt, mutation.

TABLE A5. Germline Mutations Identified by Sequencing and Clinical Criteria in Identified Cohort

Genetic Mutation	No. Identified by Sequencing	No. (%) Identified by Clinical Criteria
All high- or moderate-penetrance mutations	26	14 (53)
MSH2/MLH1/MSH6	9	6 (75)
BRCA1/BRCA2	8	7 (88)
CHEK2	2	1 (33)
RAD51C	1	0
BRIP1	1	0
SDHA	1	0
TP53	1	0
MITF	1	0
ATM	1	0
NBN	1	0
ERCC3	1	1*

*Patient met criteria for *BRCA* testing.