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Inherited Germline Cancer Susceptibility Gene Variants in Individuals with Non–Muscle-Invasive Bladder Cancer

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ABSTRACT

Purpose: Identification of inherited germline variants can guide personalized cancer screening, prevention, and treatment. Pathogenic and likely pathogenic (P/LP) germline variants in cancer predisposition genes are frequent among patients with locally advanced or metastatic urothelial carcinoma, but their prevalence and significance in patients with non–muscle-invasive bladder cancer (NMIBC), the most common form of urothelial carcinoma, is understudied.

Experimental Design: Germline analysis was conducted on paired tumor/normal sequencing results from two distinct cohorts of patients initially diagnosed with NMIBC. Associations between clinicopathologic features and clinical outcomes with the presence of P/LP germline variants in ≥76 hereditary cancer predisposition genes were analyzed.

Results: A similar frequency of P/LP germline variants were seen in our two NMIBC cohorts [12% (12/99) vs. 8.7% (10/115), $P = 0.4$]. In the combined analysis, P/LP germline variants were found only in patients with high-grade NMIBC (22/163), but none of the 46 patients with low-grade NMIBC (13.5% vs. 0%, $P = 0.005$). Fifteen (9.2%) patients with high-grade NMIBC had P/LP variants in DNA damage response genes, most within the nucleotide excision repair (*ERCC2/3*) and homologous recombination repair (*BRCA1, NBN, RAD50*) pathways. Contrary to prior reports in patients with NMIBC not receiving Bacillus Calmette-Guerin (BCG), P/LP germline variants were not associated with worse recurrence-free or progression-free survival in patients treated with BCG or with risk of developing upper tract urothelial carcinoma.

Conclusions: Our results support offering germline counseling and testing for all patients with high-grade bladder cancer, regardless of initial tumor stage. Therapeutic strategies that target impaired DNA repair may benefit patients with high-grade NMIBC.

Introduction

Inherited germline pathogenic and likely pathogenic (P/LP) variants can have profound implications for the development, treatment, and screening of various cancer types (1). Recent investigations have found that a substantial proportion of individuals with bladder cancer have P/LP germline variants in cancer predisposition genes, especially in DNA damage response (DDR) genes (2–4). To date, most of these studies have focused mostly on patients with locally advanced or metastatic urothelial carcinoma and the limited data available for non–muscle-invasive bladder cancer (NMIBC) suggests the presence of P/LP variants may be associated with substantially worse clinical outcomes (5). Yet, the influence of P/LP germline variants in patients treated with Bacillus Calmette-Guerin (BCG) immunotherapy, the most effective and commonly used treatment for high-grade NMIBC, is unknown. Thus, the prevalence and significance of P/LP variants in patients with NMIBC remain poorly defined.

NMIBC is the most common form of urothelial carcinoma accounting for more than 70% to 80% of the estimated 573,278 individuals diagnosed with bladder cancer worldwide each year (6–8). Current risk

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Translational Relevance

We conducted a germline analysis of ≥76 genes with known hereditary cancer predisposition association in two cohorts of patients with non–muscle-invasive bladder cancer (NMIBC), the most common form of urothelial carcinoma. We found a high rate of pathogenic and likely pathogenic (P/LP) germline variants for patients with high-grade NMIBC at a similar prevalence to patients with locally advanced and metastatic urothelial cancer, especially in DNA damage repair (DDR) genes. In contrast, no P/LP germline variants were seen in patients initially diagnosed with low-grade NMIBC, supporting distinct pathogenesis between high- and low-grade bladder cancer. Our results support offering germline counseling and testing for patients with high-grade bladder cancer, regardless of clinical stage. Our results also suggest that Bacillus Calmette-Guerin immunotherapy may be protective against the adverse clinical outcomes previously reported for patients with NMIBC with P/LP germline variants in DDR genes. The high prevalence of both somatic mutations and P/LP germline variants in DDR genes in high-grade NMIBC support investigations into therapeutic targeting of impaired DNA repair mechanisms.

stratification schemas that rely on clinicopathologic features cannot accurately predict which patients with NMIBC will experience recurrence or progression to muscle-invasive disease (9). To better understand the biology of NMIBC, our group and others have recently reported on the somatic mutational spectrum of NMIBC, identifying a high rate of somatic DDR gene alterations and high tumor mutational burden in high-grade NMIBC (10, 11). While somatic alterations are important determinants of biologic behavior in NMIBC, we hypothesized that P/LP germline variants are also of biologic and clinical importance. We therefore sought to determine the prevalence of P/LP germline variants in patients with NMIBC and to investigate their impact on clinically relevant outcomes.

Materials and Methods

Patient cohorts

All patients provided written informed consent to an institutional review board (IRB)–approved prospective protocol (NCT01775072) for tumor and matched normal DNA sequencing via MSK–Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT), a clinical next-generation sequencing platform that is FDA-authorized to identify somatic genetic variants in over 341 cancer-related genes (12). Peripheral blood samples were used for matched germline DNA sequencing. Participating patients consented to receive the results of their somatic mutational profile. This study was performed in accordance with Declaration of Helsinki and following IRB approval [Memorial Sloan Kettering Cancer Center (MSKCC; New York, NY) protocols 16–1249 and 17–420], secondary germline analysis was conducted in anonymous fashion. Briefly, detailed clinical data, including self-reported ancestry, religion, and race, was abstracted for each patient record. Baseline demographics, social history, family cancer history, and personal cancer history were obtained using a structured clinical assessment and manual review of electronic medical records. After clinical annotation, sequence data were assigned a unique study identifier and irretrievably delinked from personal identifiers before germline variant calling and analysis was performed in a permanently anonymized fashion. Patients included in the current study were also included in a previous germline analysis report by our group (2), but limited details on NMIBC treatment and clinical annotation were available in that report.

Original NMIBC cohort

This cohort comprised the first 99 patients at MSKCC with newly diagnosed NMIBC to undergo clinical MSK-IMPACT tumor genomic profiling within our CLIA-certified lab. These patients' data were originally analyzed to characterize the somatic genomic landscape of NMIBC (10), and they were unselected for risk of inherited cancer predisposition syndromes. All patients in this cohort underwent evaluation, treatment, and follow-up at MSKCC starting at the time of their initial diagnosis. As previously described, all patients in this cohort underwent evaluation, treatment, and follow-up at MSKCC starting at the time of their initial diagnosis. Restaging TURBT was performed in all high-grade stage T1 (HGT1) tumors to confirm that the detrusor muscle was uninvolved. A board-certified genitourinary pathologist (H. Al-Ahmadie) reviewed representative hematoxylin and eosin slides to confirm grade and stage of all index NMIBC tumors. Treatment and management were at the discretion of the treating urologic oncologist at MSKCC. Patients treated with BCG immunotherapy received six weekly full doses of TICE BCG. Patients were followed at MSKCC every 3 months by cystoscopy and urine cytology for the first year, then every 3 to 6 months.

Expanded NMIBC cohort

This second cohort comprised 115 patients with NMIBC at initial diagnosis. While 41% (47/115) of these patients were evaluated and treated at MSKCC for their initial tumor in the same fashion as the original NMIBC cohort, the other patients were initially managed for their NMIBC at an outside hospital prior to referral to MSKCC upon disease recurrence and/or progression. Thus, the expanded NMIBC cohort is enriched for patients exhibiting poor clinical outcomes, as they were offered clinical sequencing of tumor tissue at the discretion of their treating physician to determine eligibility for targeted therapy clinical trials or following progression to locally advanced or metastatic disease, among other reasons. To reduce the risk of potential understaging and falsely including a patient who actually had muscle-invasive disease in our NMIBC cohort, only patients with an initially non–muscle-invasive tumor (Tis, Ta, or T1 with uninvolved muscularis propria in the specimen) that was confirmed by a re-staging transurethral resection (TUR) or at least one tumor-free follow-up cystoscopy prior to a progression event were included (13).

BCG treatment details

All BCG-treated patients included in our outcomes analysis had high-grade NMIBC and received at least five of six weekly doses of BCG with or without maintenance therapy. Two patients received less than three instillations of induction BCG (stopped due to toxicity) and were considered non-BCG treated.

Primary muscle-invasive bladder cancer/metastasis comparison cohort

This cohort included 169 patients who initially presented with *de novo* metastatic urothelial bladder cancer or whose initial bladder tumor invaded into or beyond the muscularis propria (clinical stage ≥T2) and had no history of upper tract urothelial carcinoma (UTUC) or NMIBC (13). These patients were offered clinical sequencing of tumor tissue at their treating physician's discretion for various clinical purposes.

Variant interpretation

Germline sequencing data (BAM file) generated from the paired tumor/normal MSK-IMPACT clinical assay were analyzed in accordance with the recommendations of the American College of Medical Genetics and Genomics (ACMGG; Bethesda, MD; ref. 14). The analysis focused on germline variants in ≥76 genes with a known hereditary cancer predisposition association, for which all coding regions were sequenced in both germline normal and tumor tissue. As previously described (2), both automated computational pipelines and manual curation were used to ensure optimal germline variant calling. The potential clinical significance of identified variants was assessed by evaluating association with genetic disease as determined by reviewing the literature and online databases in conjunction with established bioinformatics pipelines. Germline variants were initially prioritized using the Pathogenicity of Mutation Analyzer (PathoMAN) classification tool that automates germline genomic variant curation in an unbiased and efficient manner (15). To further increase robustness, germline variants were also manually curated by a member of the MSKCC Niehaus Center for Inherited Cancer Genomics research team with expertise in clinical and molecular genetics according to ACMGG criteria (14). Only P/LP germline variants were included in this analysis; variants of unknown significance were reviewed but were not reported or analyzed. Biallelic inactivation through LOH was determined by the FACETS algorithm (16). P/LP variants were classified at the gene level as having high penetrance [relative risk (RR) of >4], moderate penetrance (RR of 2–4), low penetrance (RR < 2), uncertain penetrance, or association with an autosomal recessive condition (1). Classification was performed at the variant level for *CHEK2, APC*, and *ERCC3*: *APC* p.Ile1307Lys and *CHEK2* p.Ile157Thr were considered low penetrance and the *ERCC3* p.Arg109X, uncertain penetrance (17).

Statistical analysis

Clinical characteristics were compared between P/LP groups using Fisher exact tests or χ^2 tests for categorical variables, and Wilcoxon rank-sum for continuous variables. As clinical data were analyzed in anonymized fashion, event or censoring times for recurrence-free survival (RFS), biologic progression-free survival (bPFS), clinical PFS (cPFS), and time to development of UTUC were provided as intervals. Kaplan–Meier method was used to estimate probabilities for RFS, bPFS, cPFS, and time to development of UTUC, and log–rank tests were used to test for differences between groups. The icenReg R package was used to fit Cox proportional hazards models for interval censored data and identify univariable associations between P/LP germline variants and outcomes of interest. Median follow-up was estimated as the median minimal follow-up time of the censoring interval for those who did not have a progression event (18). Recurrence was defined as histologically proven urothelial cancer. Biologic progression was defined as the development of secondary muscle-invasive bladder cancer (MIBC; tumor stage ≥T2) or metastasis. As radical cystectomy is a clinically meaningful event that is often performed before biologic progression, we also analyzed cPFS as a composite endpoint of radical cystectomy and/or biologic progression, whichever occurred first. RFS, bPFS, and cPFS were calculated from the time of pretreatment TUR until the event of interest. Analyses on BCG treatment were restricted to patients with BCG-naïve, high-grade NMIBC who received at least five of six weekly doses of BCG with or without maintenance therapy. Time to UTUC development was calculated from initial diagnosis of NMIBC until histologic proven urothelial cancer of the ureter or renal pelvis/calyxes. All analyses were conducted in R version 4.1.0.

Data availability statement

The data generated in this study are available within the article and its supplementary data files. Additional data are available upon reasonable request from the corresponding author.

Results

Patient and tumor characteristics

We identified 214 patients who presented with NMIBC as their initial urothelial carcinoma diagnosis for whom germline analysis was performed (Supplementary Fig. S1). Patient and clinicopathologic features for the original and expanded NMIBC cohorts are in included in **Table 1**. As expected, patients within the original cohort were representative of the demographics and treatment outcomes of a typical NMIBC patient population (9, 10), whereas patients within the expanded cohort were enriched for poor clinical outcomes, with out of 115 patients, 66 progressed to secondary MIBC or metastasis and 31 developed subsequent UTUC (**Table 1**). The median lower bound of the censoring interval (a proxy for median follow-up) among those who did not progress was 50 months. As there was a similar frequency of P/LP germline variants in the original and expanded cohorts [12% (12/99) vs. 8.7% (10/115), $P = 0.4$; **Table 1**], these cohorts were analyzed together to investigate the significance of P/LP germline variants in patients with NMIBC.

Frequency and characterization of P/LP variants in patients with NMIBC

Of the 214 patients initially diagnosed with NMIBC, 22 (10%) had a P/LP variant in a gene associated with cancer predisposition (**Table 2**; **Fig. 1A**). Similar to prior reports in patients with locally advanced and metastatic urothelial cancers (2–4), we found a high frequency of P/LP DDR germline variants in patients with NMIBC (15 of 22, 68%; **Fig. 1A**). Within the nucleotide excision repair (NER) pathway, 2 patients had P/LP germline variants in *ERCC2* with either LOH or a somatic mutation in the second allele, suggesting that these variants likely contributed to bladder cancer development (**Table 2**; **Fig. 1B**). 2 patients carried germline *ERCC3* p.R109X alterations, a known founder mutation in the Ashkenazi Jewish population known to be associated with a moderate risk of breast cancer (17). In the homologous recombination repair (HRR) pathway, 3 patients had high-penetrance, potentially actionable *BRCA1* variants. No *BRCA2* variants were seen in patients with NMIBC despite germline *BRCA2* variants being associated with advanced urothelial carcinoma (2–4). Although Lynch syndrome is associated with both bladder cancer and UTUC (19), there were no P/LP variants in mismatch repair genes in our NMIBC cohort.

P/LP germline variants in non-DDR genes included 2 patients with fumarate hydratase (p.Lys477dup) mutations, but this variant is not associated with hereditary leiomyomatosis and renal cell cancer syndrome (**Table 2**; **Fig. 1B**; ref. 20). In addition, 4 patients carried a low-penetrance *APC* p.I1307K Ashkenazi Jewish population founder variant associated with a 1.5- to 2.2-fold increased relative risk of developing colon cancer for which the National Comprehensive Cancer Network recommends an enhanced screening colonoscopy schedule (21). All 3 patients with LOH of *APC* p.I1307K underwent radical cystectomy following BCG failure (2 progressed to secondary MIBC), but the significance of this observation remains unclear. Furthermore, the tumor sequenced for somatic mutational calling to determine biallelic inactivation in the expanded NMIBC cohort was not always from the index NMIBC tumor so further investigation into these findings are warranted.

Table 1. Clinical demographics and pathologic characteristics of the original and expanded NMIBC cohorts.

Characteristic	Original cohort, *n* = 99	Expanded cohort, *n*= 115	*P*
Age at diagnosis			
≤55	18 (18%)	34 (30%)	**0.034**
>55, ≤70	39 (39%)	50 (43%)	
>70	42 (42%)	31 (27%)	
Female sex	24 (24%)	25 (22%)	0.7
Ethnicity			
African-American	1 (1.0%)	3 (2.6%)	**0.036**
Asian	3 (3.0%)	2 (1.7%)	
White	95 (96%)	103 (90%)	
Unknown	0 (0%)	7 (6.1%)	
History of smoking	66 (67%)	80 (70%)	0.6
Ashkenazi Jewish	30 (30%)	23 (20%)	0.082
Family history of urothelial cancer	4 (4%)	8 (7%)	0.4
History of second cancer			
None	75 (76%)	83 (72%)	0.6
Breast	5 (5.1%)	3 (2.6%)	
Multiple cancer types	1 (1.0%)	3 (2.6%)	
Other	7 (7.1%)	7 (6.1%)	
Prostate	11 (11%)	19 (17%)	
Tumor stage			
T1	36 (36%)	46 (43%)	0.2
Ta	56 (57%)	48 (44%)	
Tis	7 (7.1%)	14 (13%)	
Unknown	0	7	
Tumor grade			
High	74 (75%)	89 (81%)	0.3
Low	25 (25%)	21 (19%)	
Unknown	0	5	
Number of tumors			
Single	57 (58%)	58 (64%)	0.4
Multiple	41 (42%)	33 (36%)	
Unknown	1	24	
Tumor size			
Small (<3 cm)	62 (63%)	46 (59%)	0.6
Large (≥3 cm)	36 (37%)	32 (41%)	
Missing	1	37	
Concurrent CIS			
No	67 (68%)	57 (56%)	0.10
Yes	32 (32%)	44 (44%)	
Unknown	0	14	
Initial tumor management			
Cystectomy	4 (4.0%)	5 (4.3%)	0.080
BCG	65 (66%)	92 (80%)	
Intravesical chemotherapy	10 (10%)	7 (6.1%)	
Observation	20 (20%)	11 (9.6%)	
Any BCG	73 (74%)	101 (88%)	**0.008**
Maintenance BCG			
Yes	4 (4.0%)	26 (23%)	<**0.001**
Unknown	0	2	
Progression to secondary MIBC	92 (95%)	28 (30%)	
Yes	5 (5.2%)	66 (70%)	
Unknown	2	21	
UTUC diagnosis	5 (5.1%)	31 (27%)	
Any germline P/LP variant	12 (12%)	10 (8.7%)	
P/LP germline variant in DNA damage repair gene	9 (9.1%)	6 (5.2%)	

Abbreviation: CIS, carcinoma *in situ*.

Association of P/LP germline variants with patient and tumor characteristics of the initial NMIBC

To better define which patients with NMIBC should be considered for germline testing and counseling, we compared patient demographics and initial clinicopathologic tumor characteristics between the 22 patients with P/LP variants and the 192 patients without. The presence of germline alterations overall was associated with having high-grade disease (75% no P/LP variant vs. 100% P/LP variant, $P = 0.005$) and multiple tumors at initial diagnosis (64% single tumor no P/LP variant, 38% single tumor P/LP variant, $P = 0.023$, **Table 3**); the same associations were also true specifically for DDR variants (Supplementary Table S1).

Interestingly, all 22 P/LP germline variants were found in patients with high-grade NMIBC ($n = 163$), with none of the 46 patients with low-grade Ta (LGTa) tumors having a P/LP germline variant ($P = 0.005$, **Fig. 2**). The risk of progression for patients with low-grade NMIBC is usually ≤2% (9), but inclusion of the expanded NMIBC cohort resulted in our study being uniquely enriched for patients with LGTa tumors who experienced progression events. In total, 25 of 46 patients with an initial LGTa tumor eventually progressed to ≥T1 and/or high-grade disease, 13 of whom eventually developed MIBC/metastatic disease [5-year PFS 90%; 95% confidence interval (CI), 80–100]. Therefore, the germline factors analyzed here are unlikely to contribute to disease progression in low-grade NMIBC.

As P/LP variants were only found in patients with high-grade NMIBC, we repeated our analysis comparing patients with and without P/LP variants among this subset to better risk-stratify patients for germline screening. Within this group, we found no differences in patient demographics or clinicopathologic tumor characteristics between those with any P/LP germline variants and those without (**Supplementary** Table S2), or those with versus without DDR variants (**Table 3**).

We next sought to determine whether there was any difference in the frequency of P/LP germline variants between those initially diagnosed with high-grade NMIBC ($n = 163$) versus patients who had muscle-invasive or metastatic bladder cancer at initial diagnosis ($n = 169$). We found no difference in the proportion of P/LP germline variants overall [13% (22/163) vs. 12% (21/169), $P = 0.8$] or in DDR genes specifically [9.2% (15/163) vs. 11% (18/169), $P = 0.7$; **Fig. 2**].

Association of P/LP variants with clinical outcomes in NMIBC

Because both somatic mutations and germline SNPs in DDR genes have been associated with response to BCG (10, 13, 22, 23), we hypothesized that the presence of P/LP germline variants in DDR genes would correlate with clinical benefit from BCG. However, among patients treated with BCG ($n = 148$), we found no statistically significant difference between those with and without P/LP germline DDR variants in terms of RFS (HR 0.98 ref: no variant, $P > 0.9$), bPFS (HR ref: no variant 1.1, $P = 0.9$), or cPFS (HR ref: no variant 1.14, $P = 0.8$). One-year RFS was 62% (95% CI, 40–95) in those with a DDR variant versus 67% (95% CI, 49–90) in those without (**Fig. 3A**), the 2-year bPFS was 77% (95% CI, 57–100) in the variant group and 83% (95% CI, 77–89) in those without (**Fig. 3B**), and the 2-year cPFS was 77% (95% CI, 57–100) in the variant group and 80% (95% CI, 73–87) in those without (**Fig. 3C**).

Our group recently reported that patients with initial NMIBC tumors treated with BCG that progressed to secondary MIBC had fewer somatic *ERCC2* missense mutations than those presenting with primary MIBC, which may contribute to less clinical benefit from cisplatin-based chemotherapy (13). Therefore, we also compared the frequency of P/LP variants between patients with primary ($n = 169$)

Table 2. P/LP germline variants in hereditary cancer predisposition genes.

Variant characteristics						Patient and tumor characteristics					
Affected gene	**HGVSc**	**HGVSp**	**Penetrance**	**Biallelic status**	**DDR germline mutation**	**NMIBC cohort**	**Age (years)**	**Sex**	**Ashkenazi Jewish**	**Initial tumor stage/grade**	**Initial tumor number**
APC	c.3920T>A	p.I1307K	Low	LOH	No	Expanded	66–70	M	Yes	HGT1	Single
APC	c.3920T>A	p.I1307K	Low	LOH	No	Expanded	56–60	M	Yes	HGTa	Single
APC	c.3920T>A	p.I1307K	Low	—	No	Original	>81	M	Yes	Tis (HG)	Multiple
APC	c.3920T>A	p.I1307K	Low	LOH	No	Expanded	71–75	F	No	Tis (HG)	Multiple
ATM	c.5932G>T	p.E1978*	Moderate	—	Yes	Expanded	41–45	M	No	HGT1	Multiple
BARD1	c.1652C>G	p.S551*	Uncertain	—	Yes	Original	51–55	M	No	HGT1	Single
BRCA1	c.1687C>T	p.Q563*	High	—	Yes	Expanded	<40	M	No	HGTa	Single
BRCA1	c.68_69delAG	p.E23Vfs*17	High	—	Yes	Original	66–70	F	Yes	HGTa	Multiple
BRCA1	c.116G>A	p.C39Y	High	—	Yes	Expanded	46–50	F	No	HGT1	NA
CHEK2	c.1283C>T	p.S428F	Moderate	—	Yes	Expanded	76–80	M	Yes	HGT1	Multiple
ERCC2	c.1847G>C	p.R616P	Uncertain	LOH	Yes	Original	76–80	M	No	Tis (HG)	Multiple
ERCC2	c.2150C>G	p.A717G	Uncertain	Somatic mutation	Yes	Original	61–65	M	No	HGT1	Multiple
ERCC3	c.325C>T	p.R109*	Moderate	—	Yes	Original	71–75	M	Yes	HGTa	Multiple
ERCC3	c.325C>T	p.R109*	Moderate	—	Yes	Original	71–75	M	Yes	HGT1	Single
FH	c.1431_1433dupAAA	p.K477dup	Recessive	LOH	No	Original	66–70	M	No	HGT1	Multiple
FH	c.1431_1433dupAAA	p.K477dup	Recessive	—	No	Expanded	51–55	M	No	HGTa	Single
NBN	c.2140C>T	p.R714*	Moderate	—	Yes	Expanded	66–70	M	No	HGT1	Single
NBN	c.657_661delACAAA	p.K219Nfs*16	Moderate	—	Yes	Expanded	71–75	M	No	Tis (HG)	Multiple
RAD50	c.1270_1271delCT	p.L424Efs*7	Uncertain	—	Yes	Original	66–70	F	No	HGTa	Multiple
RAD50	c.326_329delCAGA	p.T109Nfs*20	Uncertain	—	Yes	Original	61–65	M	No	Tis (HG)	Multiple
RECQL4	c.2464-1G>C	p.X822_splice	Recessive	LOH	Yes	Original	61–65	F	Yes	HGT1	Single
SDHA	c.245_252delAGGCAGGG	p.E82Vfs*2	High	LOH	No	Original	66–70	M	No	HGTa	Multiple

Abbreviations: HGVSc, Human Genome Variation Society Coding; HGVSp, Human Genome Variation Society Protein; F, female; M, male; NA, not applicable.

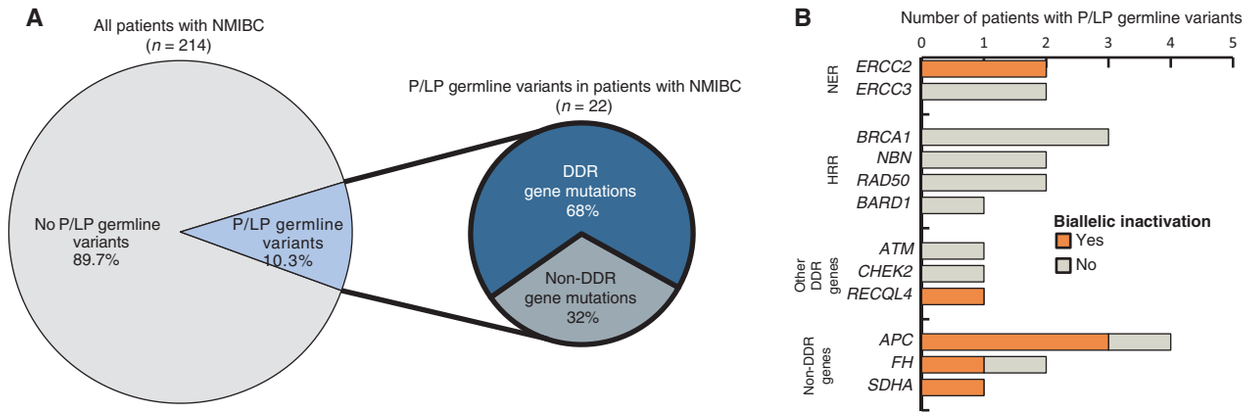

Figure 1.

P/LP germline variants in patients with NMIBC. **A,** Percentage of patients initially diagnosed with NMIBC with and without P/LP germline variants and percentage of P/LP variants in DDR genes and non-DDR genes. **B,** Frequency of P/LP variants in specific cancer susceptibility genes in patients initially diagnosed with NMIBC. Biallelic inactivation was evaluated by inferring LOH or assessing the presence of a somatic mutation in the same gene in the tumor.

and secondary MIBC after prior BCG ($n = 62$). We found no difference in the proportion of P/LP germline variants in either DDR genes [11% (18/169) versus 6.5% (4/62), $P = 0.3$] or any of the assessed germline genes [12% (21/169) vs. 9.7% (6/62), $P = 0.6$].

As UTUC is associated with Lynch syndrome, we hypothesized that patients with NMIBC who subsequently develop UTUC would have a greater portion of P/LP germline variants in mismatch repair genes. 36 patients of 214 in our cohort eventually developed UTUC, with a 5-year UTUC-free survival rate of 92% (95% CI, 88–96). However, we found no P/LP germline variants in mismatch repair genes (*MLH1, MSH2, MSH6, PMS2*) in any patient within our entire cohort. Moreover, we found no difference in risk of developing UTUC between patients with a P/LP DDR germline variant versus those without (HR 1.02 ref: no variant, $P > 0.9$; **Fig. 3D**).

Discussion

The identification of P/LP germline variants can provide guidance for personalized cancer screening, prevention, and treatment. In this large clinically annotated cohort of patients with NMIBC, we found a high rate of P/LP germline variants in patients initially diagnosed with high-grade NMIBC (13.5%), a frequency similar to that in patients with muscle-invasive and metastatic urothelial carcinoma. In contrast, we found no P/LP germline variants in patients initially diagnosed with low-grade NMIBC. While only 46 patients with LGTa were included in our study, they were enriched for adverse clinical outcomes, which suggests that the germline variants typically screened for by current multigene panels do not significantly contribute to disease initiation and progression in patients with LGTa. This distribution of P/LP germline DDR gene variants among bladder cancer grades is similar to that reported for somatic DDR gene alterations (10, 11, 23), suggesting that both somatic and germline alterations in DDR genes are important contributors to the pathogenesis of high-grade bladder cancer, but unlikely to be involved in the development of low-grade NMIBC.

Our data indicate that germline testing and counseling should be considered in any patient with high-grade bladder cancer, whether they are initially diagnosed with high-grade NMIBC, MIBC, or metastatic disease. Identification of P/LP variants in patients with high-grade NMIBC may be even more important, as most patients with NMIBC have a favorable prognosis and do not die from bladder cancer, but from other causes (24, 25). Survivors of bladder cancer have a 19% risk of developing an additional nonurothelial malignancy within 10 years of their bladder cancer diagnosis, and a 34% risk within 20 years (26). This is the highest risk of multiple malignancies among all common cancer types and more than half of those who survive their bladder cancer ultimately die from a subsequent nonurothelial malignancy (26). While shared etiologic exposures such as smoking contribute to this risk (27), P/LP variants in DDR genes may increase susceptibility to tobacco-associated DNA damage and risk of development of additional primary malignancies (28). Germline screening in patients with high grade NMIBC may afford an opportunity for the early detection of subsequent nonurothelial malignancies, along with the benefits of cascade germline testing for at-risk family members (29, 30).

Our findings may also have therapeutic implications in NMIBC. PARP inhibitors confer selective sensitivity against HRR-deficient tumors and are a standard therapy in multiple tumor types for patients with deleterious germline mutations in *BRCA1/2* and other DDR genes (31). PARP inhibition for metastatic bladder cancer remains under investigation but may have a role in genomically selected patients (32–35). While toxicity from current systemic PARP inhibitors make them unlikely treatment options for patients with NMIBC, alternative strategies that also exploit tumor vulnerabilities due to impaired DNA repair could be considered. For example, hyperthermia inhibits chemotherapy-induced poly(ADP-ribosyl)ation to a similar degree as pharmacologic PARP inhibition (36), and thus intravesical chemohyperthermia, such as hyperthermic intravesical mitomycin (37), may increase drug activity in patients with deleterious germline and somatic DDR alterations. Moreover, the high prevalence of *ERCC2* and *ERCC3* germline and somatic alterations in NMIBC suggests that selectively targeting NER-deficient tumors with intravesical cisplatin (38) or the semisynthetic DNA alkylating agent irofulven (39, 40) could provide a precision therapeutic approach for these patients.

Our results partially contrast with those of a group in Shanghai who performed targeted germline sequencing in patients with NMIBC (5). While they found a similar rate of germline DDR variants (11.3%, 8 of 71), they reported that DDR germline variants in patients with NMIBC were associated with "unfavorable outcomes" (5). In that study P/LP DDR germline variants were enriched in patients with secondary

Table 3. Association of P/LP germline variants with clinical demographics and pathologic characteristics.

	Any gene, entire cohort ($n = 214$)				DDR genes, high-grade NMIBC ($n = 163$)			
Characteristic	**Overall**	**No P/LP germline variant ($n = 192$)**	**P/LP germline variant ($n = 22$)**	***P***	**Overall**	**No P/LP germline variant ($n = 148$)**	**P/LP germline variant ($n = 15$)**	***P***
Age at initial diagnosis				>0.9				0.9
≤55	52 (24%)	47 (24%)	5 (23%)		34 (21%)	30 (20%)	4 (27%)	
>55, ≤70	89 (42%)	79 (41%)	10 (45%)		71 (44%)	65 (44%)	6 (40%)	
>70	73 (34%)	66 (34%)	7 (32%)		58 (36%)	53 (36%)	5 (33%)	
Female sex	49 (23%)	44 (23%)	5 (23%)	>0.9	30 (18%)	26 (18%)	4 (27%)	0.5
Ethnicity				0.7				0.4
African-American	4 (1.9%)	4 (2.1%)	0 (0%)		4 (2.5%)	4 (2.7%)	0 (0%)	
Asian	5 (2.3%)	4 (2.1%)	1 (4.5%)		3 (1.8%)	2 (1.4%)	1 (6.7%)	
White	198 (93%)	177 (92%)	21 (95%)		150 (92%)	136 (92%)	14 (93%)	
Unknown	7 (3.3%)	7 (3.6%)	0 (0%)		6 (3.7%)	6 (4.1%)	0 (0%)	
History of smoking	146 (68%)	130 (68%)	16 (73%)	0.6	113 (69%)	103 (70%)	10 (67%)	0.8
Ashkenazi Jewish	53 (25%)	45 (23%)	8 (36%)		39 (24%)	34 (23%)	5 (33%)	0.4
Second cancer	56 (26%)	48 (25%)	8 (36%)	0.3	39 (24%)	34 (23%)	5 (33%)	0.4
Second cancer type				0.3				0.3
Breast	8 (3.7%)	6 (3.1%)	2 (9.1%)		6 (3.7%)	4 (2.7%)	2 (13%)	
Multiple	4 (1.9%)	4 (2.1%)	0 (0%)		2 (1.2%)	2 (1.4%)	0 (0%)	
Other	14 (6.5%)	13 (6.8%)	1 (4.5%)		9 (5.5%)	8 (5.4%)	1 (6.7%)	
Prostate	30 (14%)	25 (13%)	5 (23%)		22 (13%)	20 (14%)	2 (13%)	
Family history of urothelial cancer	12 (5.6%)	10 (5.2%)	2 (9.1%)	0.4	8 (4.9%)	8 (5.4%)	0 (0%)	>0.9
Initial tumor stage				0.052				0.6
T1	82 (40%)	72 (39%)	10 (45%)		82 (51%)	74 (51%)	8 (53%)	
Ta	104 (50%)	97 (52%)	7 (32%)		57 (36%)	53 (37%)	4 (27%)	
Tis	21 (10%)	16 (8.6%)	5 (23%)		21 (13%)	18 (12%)	3 (20%)	
Unknown	7	7	0		3	3	0	
Initial tumor grade				**0.005**				
High	163 (78%)	141 (75%)	22 (100%)		163 (100%)	148 (100%)	15 (100%)	
Low	46 (22%)	46 (25%)	0 (0%)		NA	NA	NA	
Unknown	5	5	0		NA	NA	NA	
Initial number of tumors				**0.023**				0.1
Multiple	74 (39%)	61 (36%)	13 (62%)		65 (44%)	56 (41%)	9 (64%)	
Single	115 (61%)	107 (64%)	8 (38%)		84 (56%)	79 (59%)	5 (36%)	
Unknown	25	24	1		14	13	1	
Initial tumor size				0.9				0.7
Large (≥3 cm)	68 (39%)	61 (39%)	7 (37%)		60 (44%)	55 (44%)	5 (38%)	
Small (<3 cm)	108 (61%)	96 (61%)	12 (63%)		77 (56%)	69 (56%)	8 (62%)	
Unknown	38	35	3		26	24	2	
Initial concurrent CIS	76 (38%)	66 (37%)	10 (48%)	0.3	76 (49%)	69 (49%)	7 (47%)	0.8
Unknown	14	13	1		8	8	0	
Initial tumor management				0.4				0.6
Cystectomy	9 (4.2%)	8 (4.2%)	1 (4.5%)		8 (4.9%)	7 (4.7%)	1 (6.7%)	
Initial BCG	157 (73%)	138 (72%)	19 (86%)		138 (85%)	126 (85%)	12 (80%)	

(Continued on the following page)

Table 3. Association of P/LP germline variants with clinical demographics and pathologic characteristics. (Cont'd)

	Any gene, entire cohort (n = 214)				DDR genes, high-grade NMIBC (n = 163)			
Characteristic	Overall	No P/LP germline variant (n = 192)	P/LP germline variant (n = 22)	P	Overall	No P/LP germline variant (n = 148)	P/LP germline variant (n = 15)	P
Intravesical chemo	17 (7.9%)	17 (8.9%)	0 (0%)		4 (2.5%)	4 (2.7%)	0 (0%)	
Observation	31 (14%)	29 (15%)	2 (9.1%)		13 (8.0%)	11 (7.4%)	2 (13%)	
BCG	174 (81%)	154 (80%)	20 (91%)	0.4	148 (91%)	135 (91%)	13 (87%)	0.6
Induction-only BCG				0.5				0.8
Induction only	142 (67%)	125 (66%)	17 (77%)		121 (75%)	110 (75%)	11 (73%)	
Maintenance given	30 (14%)	27 (14%)	3 (14%)		26 (16%)	24 (16%)	2 (13%)	
No BCG	40 (19%)	38 (20%)	2 (9.1%)		15 (9.3%)	13 (8.8%)	2 (13%)	
Unknown	2	2	0		1	1	0	
Secondary MIBC				0.6				0.4
No progression	120 (63%)	107 (62%)	13 (68%)		87 (59%)	78 (58%)	9 (69%)	
Secondary MIBC	71 (37%)	65 (38%)	6 (32%)		61 (41%)	57 (42%)	4 (31%)	
Unknown	23	20	3		15	13	2	
UTUC diagnosis	36 (17%)	33 (17%)	3 (14%)	>0.9	25 (15%)	24 (16%)	1 (6.7%)	0.5

Abbreviations: CIS, carcinoma *in situ*; NA, not applicable.

Figure 2.
Frequency of P/LP germline variants in patients initially diagnosed with low-grade NMIBC versus high-grade NMIBC versus primary MIBC or metastatic disease. UC, urothelial carcinoma.

MIBC compared with those with "non-progressive" NMIBC [4 of 11 (36%) vs. 4 of 60 (6.7%), $P < 0.02$; 5]. As we restricted our clinical outcomes analysis to only patients treated with BCG, whereas few, if any, patients in the Shanghai study would have received BCG given the availability issues in China during their study period (5). Thus, our conflicting findings may be the result of DDR germline variants conferring worse outcomes in non–BCG-treated patients compared with those treated with BCG.

Although our study is the largest investigation to date focused on germline P/LP variants in patients with NMIBC, the overall low frequency of P/LP variants in each cancer-associated gene limits our ability to make any firm conclusions about their impact on clinical outcomes. Moreover, additional studies are needed to better understand whether germline variants would have the same implications as somatic mutations may have on therapeutic responses in bladder cancer (10, 13, 23, 41). The impact of germline variants is dependent on lineage and penetrance for each specific gene, which requires further investigation in bladder cancer (42). Our findings might also not be generalizable to the wider population of patients with NMIBC, as MSKCC is a specialized cancer referral center in the northeast United States and patients in our study were selected for tumor-normal next-generation sequencing. Our population is also enriched in Ashkenazi Jewish ancestry, a group who tend to harbor more founder germline variants than others (17). In addition, most patients in our study were male and self-reported as White. Taken together, these factors may limit the generalizability of our finding and support the need for validation in larger and more diverse multicenter prospective cohorts.

In conclusion, we found a high rate of P/LP germline variants in patients with high-grade NMIBC (13.5%), a similar frequency to that among patients with locally advanced and metastatic urothelial cancer. If validated in additional NMIBC cohorts, our results support offering germline counseling and testing for patients with high-grade bladder cancer, regardless of clinical stage. This could improve early detection of subsequent nonurothelial malignancies for patients and allow for cascade germline testing for family members potentially at risk for urothelial and nonurothelial malignancies. Finally, the high prevalence of both somatic mutations and germline variants in DDR genes support continued investigation of therapeutic strategies targeting impaired DNA repair in high-grade NMIBC.

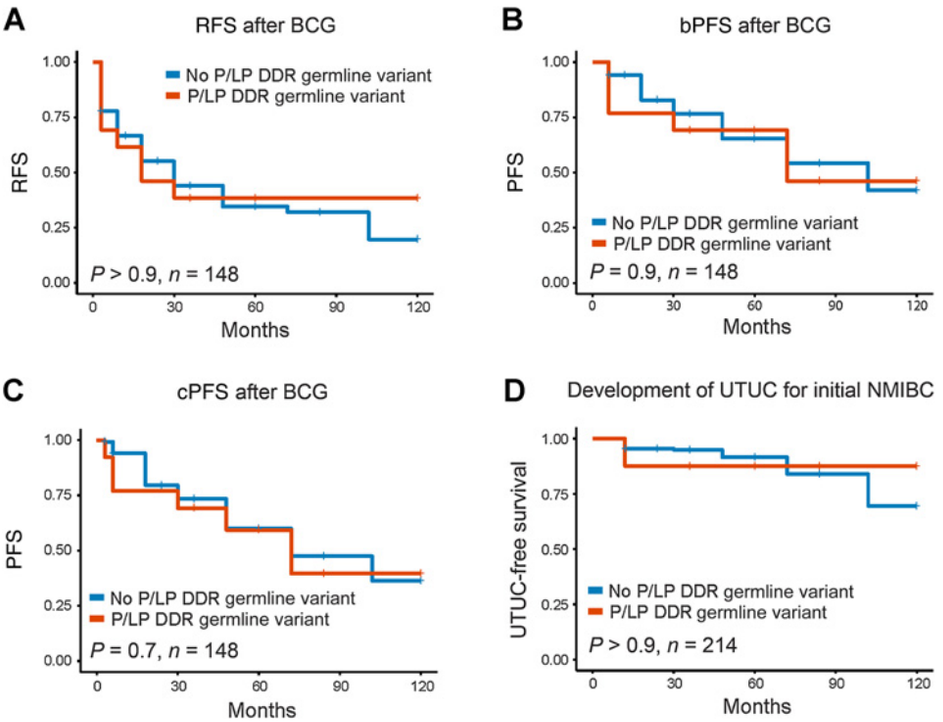

Figure 3.

Survival and risk of progression according to the presence of P/LP germline variants in DDR genes. RFS (**A**), bPFS (defined as development of tumor stage ≥ T2 or metastasis; **B**), cPFS in patients with initial high-grade NMIBC treated with BCG immunotherapy (defined as the composite endpoint of radical cystectomy and/or development of tumor stage ≥T2 or metastasis, whichever occurs first; **C**), development of UTUC in all patients with intial NMIBC (**D**). *P* values shown in figures are for HRs comparing germline variants to no germline variants in univariate interval censored Cox proportional hazards models.

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Note

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