

Prevalence of Mismatch Repair Deficiency in Primary Prostate Cancer in a Large Prospective Cohort

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Abstract

Background: Mismatch repair (MMR) deficiency and microsatellite instability are predictive biomarkers for immunotherapy response. The best approach to identify patients with such tumors is unclear in prostate cancer.

Methods: This study included 1,016 men diagnosed with primary prostate cancer during prospective follow-up of the Health Professionals Follow-up Study and Physicians' Health Study. Highest-grade/index lesions from radical prostatectomy (95%) or transurethral resections of the prostate were mounted on tissue microarrays. Scoring of immunohistochemistry for the MMR proteins MLH1, MSH2, MSH6, and PMS2 required a non-tumor internal positive control for designating deficiency. Validation was done on full sections and with PCR-based quantification of microsatellite repeats.

Results: Tumor stage was predominantly pathologically localized with a full distribution of Gleason scores. MMR tumor scoring could be performed with available internal positive control tissue in 75%–90% of cases, depending on the MMR protein. Of the 903 tumors evaluable for MSH2 protein loss, 4 tumors had loss of MSH2 (prevalence 0.4%, 95% confidence interval [CI] 0.2–1.1%), and 3 of 708 evaluable tumors had concomitant loss of MSH6 (prevalence 0.4%, 95% CI 0.1–1.2%). No tumor had loss of MLH1 or PMS2. The 4 MMR-deficient cases had higher Gleason scores, and 3 had non-zero microsatellite repeats.

Conclusions: In this nationwide prospective study, MMR deficiency was rare in primary, surgically treated prostate cancer. The low prevalence and the need for an internal positive control for this assay are feasibility concerns for unselected routine immunohistochemistry-based screening for MMR deficiency on limited tissue specimens, such as prostate biopsies.

Translational Relevance

dMMR testing is used in colon and endometrial cancer for immunotherapy eligibility and Lynch syndrome screening. The current study, in conjunction with a few high-quality prior studies of primary prostate cancer, suggests that yields of universal dMMR tumor screening with immunohistochemistry at initial diagnosis of non-metastatic prostate cancer would likely be low. Focusing dMMR testing on high-grade cancers could be one strategy to increase the yield, in addition to tumor testing for dMMR among known Lynch syndrome carriers, as not all prostate cancers in these men have dMMR. When technically feasible, next-generation sequencing is an appealing alternative that could also help detect other alterations that could become actionable.

Introduction

The mismatch repair (MMR) system can be defective (dMMR) due to germline pathogenic variants (1) or somatic alterations in *MSH2*, *MSH6*, *MSH1*, or *PMS2*, as well as 24 additional, less commonly altered genes that are part of the MMR pathway (2,3). DNA replication errors occur and manifest particularly in repeated DNA sequences, termed microsatellites, defining the phenotype of high microsatellite instability (MSI-H). dMMR and MSI-H are predictive of response to immunotherapy in several types of solid tumors (4,5). The anti-PD-1 immunotherapy agent pembrolizumab was approved by the U.S. Food and Food Administration for dMMR/MSI-H regardless of tumor type. Profound and durable responses to anti-PD-1/PD-L1 therapy in dMMR/MSI-H prostate cancer have been reported (5–8).

dMMR/MSI-H is relatively rare in prostate cancer. Estimates from previous studies differ widely, likely as a function of patient and tumor characteristics as well as biomarker testing methods. Consequently, it is unclear to date which patients with prostate cancer should best be tested for dMMR or MSI-H and which test(s) to use to best identify those patients who may benefit from immunotherapy. Some of these considerations even apply to testing tumor tissue from men with Lynch syndrome-defining germline variants, as not all prostate cancers in these men have dMMR (9).

The objective of the present study was to evaluate an immunohistochemical approach for the detection of dMMR at diagnosis, using surgical specimens from men diagnosed with prostate cancer while participating in two nationwide prospective cohort studies, and to quantify the prevalence of dMMR in this population.

Methods

Study population

This study included men who participated in two U.S. nationwide cohort studies, the Health Professionals Follow-up Study (HPFS) and the Physicians' Health Study (PHS), who were initially cancer-free at cohort enrollment and diagnosed with incident cancer during prospective follow-up. The PHS I began in 1982 as a randomized-controlled trial among 22,071 physicians aged 40–84 years. PHS II began in 1997 among 14,641 physicians aged 50 years and older; after discontinuation of randomized assignments to aspirin and micronutrients, participants were followed as a prospective cohort. HPFS is an ongoing prospective cohort study that began in 1986 when 51,529 health professionals aged 40–75 years completed a questionnaire on diet, lifestyle factors, and medical diagnoses.

The study was conducted in accordance with the U.S. Common Rule, and was approved by the institutional review boards of Brigham and Women's Hospital and Harvard T.H. Chan School of Public Health. Participants provided written informed consent by returning baseline questionnaires.

Men who were diagnosed with prostate cancer between 1982 and 2005, who underwent prostatectomy or transurethral resection of the prostate, and on whom archival formalin-fixed, paraffin-embedded tumor tissue specimens were successfully retrieved from treating hospitals were eligible for the current study.

Tumor hematoxylin/eosin slides were centrally re-reviewed by genitourinary pathologists to provide uniform evaluation of Gleason score (10) and to identify areas of high-density tumor for construction of tissue microarrays (11). At least three 0.6 mm tumor cores were sampled from each case from the same dominant tumor nodule with the highest Gleason score, in addition to one core from adjacent, histologically normal prostate tissue for a limited subset of cases.

Immunohistochemistry for MMR proteins in tumor tissue

For immunohistochemistry, 4 µm sections were mounted on FLEX slides (Dako, Glostrup, Denmark), dried at room temperature, and incubated at 60 °C for 60 min before immunostaining. Staining procedures were automated using a Leica Bond Max instrument using the following antibody clones, antigen retrieval and antibody incubation times: MLH-1 with clone ES05 (Leica), antigen retrieval ER2 for 30 min, antibody incubation for 15 min; MSH2 with clone 25D12 (Leica), antigen retrieval ER2 for 30 min, antibody incubation for 15 min; MSH6 44 (BD Biosciences),

antigen retrieval ER2 for 40 min, antibody incubation for 15 min; PMS2 with clone A16-4 (BD Sciences), antigen retrieval ER2 for 40 min, antibody incubation for 15 min.

Scoring for dMMR was performed by a senior pathology resident (M.M.N.). All cases with potential dMMR were re-reviewed by a consultant pathologist (S.P.F.). First, presence of tumor tissue cores was ascertained. The level of nuclear protein expression was scored as no staining, weak staining, or moderate/strong or diffuse staining, and any intensity of staining was subsequently considered as positive. For designating a case as dMMR, in addition to negative staining in all tumor nuclei, an internal control of positive nuclear staining for the respective MMR protein in stromal cells, lymphocytes, or benign epithelial cells of the same case was required. One case was considered to have a borderline MSH2 internal positive control but determined to be dMMR upon consensus re-review.

Validation of immunohistochemistry on full sections and evaluation of intratumoral heterogeneity

To evaluate the influence of the limited tissue area represented on tissue microarrays and validate the main staining protocol, MMR assays were repeated on informative samples from the study population as well as additional samples, all using a different MMR staining protocol. MSH2 was assayed on full sections of the dMMR cases from the study population as well as a corresponding number of MMR-intact cases. In addition, assays for all four MMR proteins were done on biopsy samples (which have a size roughly similar to TMA cores) and matching full sections of prostatectomy samples from four additional anonymized clinical cases from St. James's Hospital as well as full sections of one known dMMR case from Basel University Hospital, with tissue obtained from a transurethral resection of the prostate. All validation assays used the accredited MMR panel in clinical routine use at St. James's Hospital, using different primary antibodies and processing from what is described above. Staining was conducted using the Ventana Benchmark Ultra platform for MLH1 (Dako FLEX monoclonal mouse, clone ESO5), PMS2 (Dako FLEX monoclonal rabbit, clone GA087), MSH2 (Ventana mouse monoclonal, clone G219-1129), and MSH6 (Ventana rabbit monoclonal, clone SP93). After verifying presence of the internal positive control, intensity in and heterogeneity of tumor MMR protein expression was scored semi-quantitatively by eye by a consultant pathologist (S.P.F.).

Microsatellite instability (MSI) in tumor tissue

Tumor DNA from 4 dMMR cases and from 3 MMR-intact cases, which had been evaluable for all 4 immunohistochemical markers, was subjected to microsatellite analysis. DNA was extracted from up to two 0.6 μ m cores per high-density tumor region per case, using the QiaAmp DNA FFPE Tissue kit without modifications (Qiagen, Hilden, Germany). DNA samples from cores with a DNA yield of >4,000 ng as per NanoDrop (ThermoFisher Scientific, Waltham, MA) were additionally split into two technical replicates. The identity of biological replicates (cores) and technical replicates (DNA samples from the same core) was blinded until after initial data analysis. The 13 DNA samples were then subjected to MSI analysis using the Idylla MSI Test (Biocartis, Mechelen, Belgium) for polymerase chain reaction (PCR)-based detection of MSI-defining mutations at MSI loci in 7 genes (*ACVR2A*, *BTBD7*, *DIDO1*, *MRE11*, *RYR3*, *SEC31A*, and *SULF2*), which is known to diverge from dMMR protein status, especially beyond colorectal cancer (12).

Statistical analysis

For descriptive analyses, dMMR prevalence was defined among cases with an evaluable positive control. Confidence intervals (CIs) for proportions were based on the score test (Wilson) approach (13).

Data availability

Study data are available through an HPFS project proposal (<https://hsph.harvard.edu/research/health-professionals/resources/for-external-collaborators/>). Additional inquiries can be directed to the corresponding author.

Results

Study population

There were 1,016 study participants with prostate cancer who had MMR assessments for at least one of the four markers across 12 tissue microarrays. Median age at diagnosis was 66 years (interquartile range 62, 69). 96% were White. The vast majority of tumors were clinically localized (T1/T2, 94%). On prostatectomy, 11% were not organ-confined (pT3b, pT4, or N1). A full distribution of Gleason scores was represented, ranging from 20% score 6 (grade group 1) to 13% scores 9-10 (grade group 5).

Immunohistochemistry for MMR proteins in tumor tissue

MMR tumor scoring could be performed with available internal positive control tissue in 72%–90% of cases, depending on the MMR protein. There were no notable differences in characteristics of cases with and without tumor tissue evaluable for MSH2 (**Table 1**).

Of the 903 tumors evaluable for MSH2, four had loss of MSH2 (prevalence 0.4%, 95% CI 0.2, 1.1; **Table 2; Figure 1A–D**). Of 708 evaluable tumors, three had loss of MSH6 (prevalence 0.4%, 95% CI 0.1, 1.2), all of which also had MSH2 loss. The fourth tumor with MSH2 loss was not evaluable for MSH6 because of an absent internal positive control, and even the internal positive control for MSH2 was faint. No tumor had loss of MLH1 or PMS2 (prevalence 0.0%, 95% CI 0.0, 0.5).

The 4 dMMR tumors were localized and had Gleason scores of 3+4, 8, 8, and 9–10 (**Table 1**). Thus, 75% of dMMR cases had Gleason score of 8 or 9–10 (grade groups 4 and 5), compared to 178 of 899 MMR-intact cases (20%), a prevalence ratio of 3.8 (95% CI 2.1, 6.8). Diagnostic prostate-specific antigen levels were 4, 4, 23, and 44 ng/mL, respectively. The tumor with faint internal positive control staining for MSH2 had a Gleason score of 3+4, and the participant had a prostate-specific antigen of 23 ng/mL at diagnosis.

Validation of immunohistochemistry on full sections and evaluation of intratumoral heterogeneity

Full slide sections of the 4 dMMR tumors and of 4 MMR-intact tumors, evaluated using a different clinical-grade MSH2 protocol, showed results consistent with information obtained from the limited tissue area represented on tissue microarrays (**Figure 2A–H**). In all 8 cases, intratumoral heterogeneity was limited (**Supplementary Table 1**), leading to the same designations as dMMR or MMR-intact as based on tissue microarray spots. When comparing the 4 MMR proteins between the limited tissue area represented in 4 diagnostic biopsies to the full slide sections from 4 corresponding prostatectomies, intratumoral heterogeneity in intact MMR protein expression was limited (**Figure 2I–P; Supplementary Table 1**). Similarly, in a case with known dMMR for MSH2/MSH6, intratumoral heterogeneity across the full slide was limited (**Figure 2Q–T; Supplementary Table 1**).

MSI in tumor tissue

Of the four dMMR tumors, 7 DNA samples were subjected to the PCR-based test, which included two technical replicates (DNA samples) from cores of two cases and two biological replicates (cores) from one case. All but one sample had non-zero PCR results for at least 3 of the 7 PCR loci (**Table 3**). The one sample with all-zero results was the case with a faint internal positive control staining for MSH2 protein and non-evaluable MSH6. Only one case (tumor 3, **Table 3**) was designated MSI-high per the manufacturer's algorithm (12). The six DNA samples from MMR-intact tumors, including two biological and two technical replicates, had all-zero results at all loci. Reproducibility between the two sets of technical replicates (identical DNA) for non-zero PCR results was high. In contrast, reproducibility between the one set of biological replicates (cores from the same case, tumor 2; **Table 3**) with non-zero scores was noticeably lower.

Discussion

In this nationwide prospective study primary, surgically treated prostate cancer, the prevalence of dMMR was only 0.4% (95% CI 0.2, 1.1). This corresponds to 4 cases in our large series and an estimated 1 in 250 patients with dMMR tumors (1 in 91 to 1 in 500, based on the 95% CI). Up to one quarter of tumor samples was not evaluable for some of the MMR proteins. A PCR-based commercial test for MSI in colorectal cancer detected evidence of MSI in three of the four dMMR cases, but also demonstrated less than optimal reproducibility between biological replicates.

The observed prevalence of dMMR in the present study is slightly lower than, but statistically compatible with, the estimates reported in the literature to date (**Table 4**) (14–20). The prevalence of dMMR ranged from 0.7% to 1.2% in the studies that focused on localized prostate cancer, used internal positive controls, and validated presumed dMMR with additional assays (14–17). Studies of MSI and mutations in MMR genes using sequencing assays, including cell-free DNA in the blood, predominantly included patients with metastatic prostate cancer and reported higher prevalence of dMMR and MSI-high (2,7,21–23).

Two major drivers likely underlie differences in observed prevalence. First, unlike our study, other studies to date were hospital-based and tended to enroll patients with advanced-stage or high-grade tumors, including studies that predominantly or exclusively evaluated samples of tumors that had progressed to metastatic castration-resistant prostate cancer or were *de novo* metastatic (**Table 4**). The current study and previous studies strongly suggest that dMMR may be more common in higher-grade and advanced-stage prostate cancers. In our study, all three tumors with an unequivocally positive internal control had Gleason scores of 8 or higher. Second, studies have used a number of different assays and have defined the outcome by dMMR, MSI, and/or high tumor mutational burden. False-positive results occur with all types of assays for different reasons, such as sequencing errors and variants of unknown significance affecting sequencing assays and poor sensitivity or specificity of unvalidated primary antibodies and antigen retrieval protocols affecting immunohistochemical assays (24).

The antibodies and protocols used for our study were fully validated for clinical use, frequently assessed and approved under an international External Quality Assurance scheme, and fully accredited by the Irish National Accreditation Board. However, the antibodies have not specifically been validated for prostate cancer, and it is possible that alternative clones, platforms, or protocols could give different results. We employed an orthogonal, clinical-grade automated staining protocol for validation on full sections of the dMMR cases from our population-based study and additional clinical cases, both dMMR and MMR-intact (**Figure 2**). This approach confirmed our main results. Moreover, intratumoral heterogeneity in these cases was limited to an extent that did not lead to differences in scoring between the limited tissue areas represented on tissue microarrays and prostate biopsies. One explanation for limited intratumoral heterogeneity in dMMR are early, clonal losses (14). While these observations underscore the validity of the main results in the current study, they do not rule out the presence of significant intratumoral heterogeneity in other cases, which could affect scoring of biopsy tissue in clinical practice.

To ensure specificity of dMMR designations in the current study, we required the visible presence of the respective MMR protein in specific non-tumor cells. With the small tissue specimens available on tissue microarrays, 10% of tumors in our study could not be evaluated for MSH2 because no internal positive control was visible. Tumors not evaluable for MSH2 were not systematically different in terms of grade or stage from those that were evaluable. At 25–28%, dropout was even higher for MSH6, MLH1, and PMS2, likely driven by differences in antibody performance or assay optimization for use on our archival samples. Tissue processing and fixation was done at healthcare facilities nationwide, and this heterogeneity may have contributed to the extent of unevaluable cases encountered in the present study. While prostate biopsies, a potential setting for dMMR screening, are also limited in tissue size, using full sections instead of tissue microarrays may also have made more cases evaluable. In addition, future studies using full sections of a much larger set of dMMR cases would enable an assessment of whether loss of MMR proteins in all tumor nuclei needs to be required, as it was done in the current study, or if dMMR only seen in some areas of the tumor might still be meaningful in prostate cancer.

A key consequence of dMMR is MSI. When we subjected the dMMR tumors and a small set of MMR-intact tumors to a commercial PCR-based assay for MSI that was designed for colorectal cancer, we found some evidence for MSI in three of the four samples, but not in all three MMR-intact tumors and notably not the one case with a faint internal positive control staining. We did not alter the dMMR designation for this tumor given that the MSI assay is not validated for prostate cancer, was run with limited archival DNA input below manufacturer recommendations, and had imperfect reproducibility between the two biological replicates of one dMMR tumor. It is possible that differences in cancer risk factors also contribute to the observed prevalence of dMMR in diagnosed tumors. dMMR is likely an early event in prostate cancer, given its clonality (7,14,20), and could be expected to be etiologically distinct from other subtypes with distinct risk factor profiles, such as prostate cancer with ETS fusions (8). In our study population, current smoking at cancer diagnosis was relatively uncommon (25), and smoking is an established risk factor of dMMR at least in colorectal cancer (26).

Limitations of the current study include the reliance on tissue microarrays and on surgical samples, which could select for a specific subset of patients with localized prostate cancer. However, prostatectomy was used in a less risk-adapted fashion during the decades when cases in our study were diagnosed than current guidelines recommend, and no indicators of potential dMMR status were known clinically that could have influenced treatment choice; thus, the potential for relevant selection bias appears small. Only four protein markers were evaluated in this study, and other protein markers define dMMR in rare cases (27); however, the markers used in this study define dMMR most commonly in prostate cancer (2,7,14,22). Additionally, testing for MSI was performed on only in a subset of the cohort, allowing no formal assessment of the sensitivity of the immunohistochemical assay. Lastly, the racial composition of our study reflects that of health professionals at cohort enrollment but not of the general U.S. population. At least for tumor MSI scores, no major difference across thousands of samples and tumor types has been found between European-ancestry and African-ancestry patients (28).

dMMR testing is performed in colon and endometrial cancers serving dual purposes: assessing for eligibility for treatment with immunotherapy and screening for Lynch syndrome. While the risk of prostate cancer is increased in Lynch syndrome carriers, far from all prostate cancers among carriers are due to Lynch syndrome, and many of these tumors do not have evidence of dMMR (9). Either way, the current study, in conjunction with prior studies of primary prostate cancer, suggests that yields of universal dMMR tumor screening at initial diagnosis of non-metastatic prostate cancer would likely be low. Focusing dMMR testing on high-grade cancers could be one strategy to increase the yield of early identification of patients for investigational approaches to immunotherapy or who have an approved indication for immunotherapy with later disease progression. However, the extent to which the MMR status of a potentially tested primary tumor and a later metastasis can be expected to be concordant needs to be empirically established (8). When technically feasible, next-generation sequencing is an appealing alternative that could also help detect other actionable alterations.

In summary, dMMR was rare in primary, surgically treated prostate cancer. This low prevalence is in line with studies of localized prostate cancer that ensured specificity of immunohistochemical dMMR determination but contrasts with hospital-based studies of dMMR and MSI that may have represented tumors with certain clinical features, such as higher grade. The low prevalence and, for the tested assay, the need for an internal positive control for reliable scoring raises feasibility concerns for routine immunohistochemistry-based screening for MMR deficiency of all prostate cancers on limited tissue specimens, such as prostate biopsies.

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Figure Legends

Figure 1. Representative images of immunohistochemical staining for MMR proteins. Columns are tumors (patients) 1 through 7, rows are MMR proteins PMS2 (first row, **A**), MLH1 (second row, **B**), MSH2 (third row, **C**), and MSH6 (fourth row, **D**). Tumors 1–4 were scored dMMR based on absent MSH6 nuclear staining in tumor cells despite intact nuclear staining in other cells of the same case. Tumors 1–3 also demonstrate dMMR by loss of MSH2. Tumor 4 had faint positive control staining for MSH6 and is unevaluable for MSH2. Tumors 5–7 are MMR-intact. Photomicrographs were taken at 40X optical magnification. The scale bar is 50 μ m. Arrows point to tumor epithelial cells, unless tumor predominates in the image.

Figure 2. Validation of MMR immunohistochemistry using an orthogonal, clinical-grade assay on full sections, and evaluation of the extent of intratumoral heterogeneity in MMR protein loss. Arrows denote tumor epithelial cells, unless tumor predominates in the image; arrowheads denote internal positive controls in non-tumor cells. Images were taken at 20X or 40X optimal magnification, and scale bars represent 50 μ m. The first two rows show full sections corresponding to the tissue microarrays spots in Figure 1 for dMMR cases (**A**, tumor 1 in Figure 1; **B**, tumor 2; **C**, tumor 3; **D**, tumor 4) as well as MMR-intact cases (**E**, tumor 5; **F**, tumor 6; **G**, tumor 7; **H**, additional full section of an MMR-intact tumor as per tissue microarray). Note the absence of nuclear staining in dMMR cases in **A** (tumor 1) and **D** (tumor 4) with some cytoplasmic staining. The third and fourth rows show biopsy (**I–L**) and corresponding prostatectomy full sections (**M–P**) for one of four hospital-based cases, all MMR-intact, stained for PMS2 (**I/M**), MLH1 (**J/N**), MSH2 (**K/O**), and MSH6 (**L/P**). The fifth row shows full sections of a hospital-based case with known MSH2/MSH6 dMMR (**Q**, PMS2; **R**, MLH1; **S**, MSH2; **T**, MSH6). Scoring results for all comparisons to full sections are in **Supplementary Table 1**.

Table 1. Patient and tumor characteristics among all participants with prostate tumor tissue evaluated, by MSH2 status, Health Professionals Follow-up Study and Physicians' Health Study, 1982–2005.^a

	MSH2 deficient MMR intact^b Internal control absent		
Tumor count	4	899	99
Gleason score			
<7	0 (0%)	176 (20%)	24 (24%)
3+4	1 (25%)	330 (37%)	33 (33%)
4+3	0 (0%)	213 (24%)	21 (21%)
8	2 (50%)	61 (7%)	13 (13%)
9-10	1 (25%)	117 (13%)	8 (8%)
Unknown	0	2	0
Clinical TNM stage			
T1/T2	4 (100%)	825 (94%)	93 (95%)
T3	0 (0%)	30 (3%)	3 (3%)
T4/N1	0 (0%)	7 (1%)	1 (1%)
M1	0 (0%)	17 (2%)	1 (1%)
Unknown	0	20	1
Pathology/RP TNM stage			
pT2	3 (100%)	593 (71%)	53 (65%)
pT3/T3a	0 (0%)	142 (17%)	22 (27%)
pT3b	0 (0%)	61 (7%)	4 (5%)
pT4/N1	0 (0%)	34 (4%)	2 (2%)
Unknown	1	69	18

^a Data shown are count (percent). Of the 1,016 total participants, 14 participants are not shown in this table as they did not have tissue cores remaining after staining the tissue microarrays for MSH2 but had tumor tissue for one or more of the other three biomarkers.

^b No MSH2-intact tumor was deficient for MSH6, MLH1, or PMS2.

Table 2. dMMR scoring results in prostate tumor tissue, Health Professionals Follow-up Study and Physicians' Health Study, 1982–2005.

Marker	Tumors	Internal control present ^a		Deficient ^b	
		Count	% (95% CI)	Count	% (95% CI)
MLH1	990	747	75 (73, 78)	0	0.0 (0.0, 0.5)
MSH2	1002	903	90 (88, 92)	4	0.4 (0.2, 1.1)
MSH6	952	708	74 (72, 77)	3	0.4 (0.1, 1.2)
PMS2	974	703	72 (69, 75)	0	0.0 (0.0, 0.5)

^a The internal positive control was evaluable.

^b Prevalence of dMMR was defined among evaluable tumors with the internal positive control present.

Table 3. MSI testing with a PCR-based assay in dMMR and MMR-intact prostate tumors, Health Professionals Follow-up Study and Physicians' Health Study. Higher values suggest MSI.

Tumor	dMMR ^a	Core ^b	Sample ^c	PCR result for microsatellite locus by gene						
				<i>ACVR2A</i>	<i>BTBD7</i>	<i>DIDO1</i>	<i>MRE11</i>	<i>RYR3</i>	<i>SEC31A</i>	<i>SULF2</i>
1	MSH2, MSH6 I	a	a	0	0.01	0.03	0.08	0	0	0
1	MSH2, MSH6 I	b	b	0.01	0.01	0.11	0.03	0	0	0
2	MSH2, MSH6 I	a	a	0.02	0.01	0.97	0.05	0.01	0	0.12
2	MSH2, MSH6 I	b	b	0.02	0.04	0.91	0.06	0.01	0.01	0.01
2	MSH2, MSH6 II	a	a	0	0.01	0.12	0.02	0	0	0
3	MSH2, MSH6 I	a	a	1	0.64	0.95	1	1	0.99	0.35
4	MSH2, - ^d	I	a	0	0	0	0	0	0	0
5	Intact	I	a	0	0	0	0	0	0	0
6	Intact	I	a	0	0	0	0	0	0	0
7	Intact	I	a	0	0	0	0	0	0	0
7	Intact	I	b	0	0	0	0	0	0	0
7	Intact	II	a	0	0	0	0	0	0	0
7	Intact	II	b	0	0	0	0	0	0	0

^a MMR proteins that this case was deficient for.

^b First or second tumor core (biological replicate).

^c First or second DNA sample (technical replicate) from the tumor core.

^d MSH6 unevaluable.

Table 4. Prevalence of dMMR in primary prostate cancer samples, based on immunohistochemistry for MLH1, MSH2, MSH6, and PMS2 reported in selected prior publications.

Year	Author	Setting	Disease state	Tissue	Sample ^a	Control ^b	Validation ^c	dMMR / N	%
2017	Guedes (14)	Hospital, USA	Localized	Prostatectomy	TMA, full slides ^d	Yes	Multiple	14 / 1,176	1.2%
2019	Albero-González (18)	Hospital, Spain	Presumed localized	Prostatectomy	TMA	Yes	No	16 / 200	8%
2020	Sharma (19)	Hospital, USA	Localized	Prostatectomy	TMA	No	No	50 / 220	23%
2020	Fraune (20)	Hospital, Germany	Advanced, limited detail	Prostatectomy	TMA, full slides ^d	Yes	Tumor PCR	7 / 200	3.5%
2021	Kagawa (15)	Hospital, Japan	Localized	Prostatectomy	Full slides	Yes	Multiple	4 / 337	1.2%
2023	Zhang (16)	Hospital, China	Predominantly localized	Mostly prostatectomy	Full slides	Yes	Tumor sequencing	8 / 1,141	0.7%
2023	Oka (17)	Hospital, Japan	Localized	Prostatectomy	TMA	Yes	Germline	1 / 129	0.8%
–	The current study	Population-based, USA	Localized (94%)	Prostatectomy (95%)	TMA	Yes	Tumor PCR	4 / 1002	0.4%

^a Sampling approach used: Tissue microarray (TMA) or full slides.

^b Whether an internal positive control was required for designating a case as dMMR.

^c Whether validation with a DNA-based test was performed. Includes PCR assays for MSI, promoter methylation assays, and sequencing assays for somatic and/or germline mutations in MMR genes.

^d In a subset of cases. Guedes *et al.* screened all cases for MSH2 on tissue microarrays and only those with MSH2 loss for the other three proteins on full slides.

Figure 1

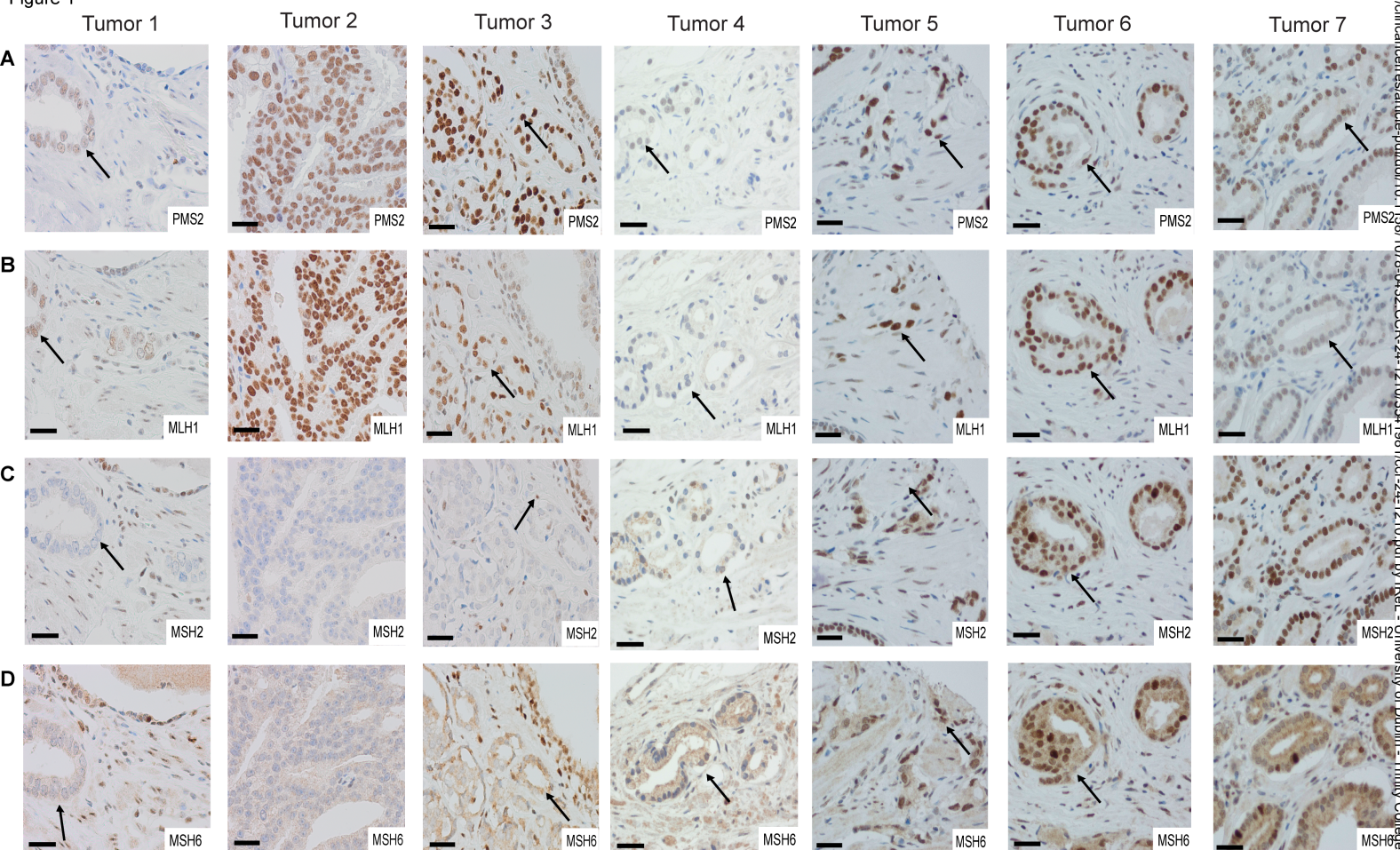


Figure 2

