Prevalence of DNA repair gene mutations in localized prostate cancer according to clinical and pathologic features: association of Gleason score and tumor stage

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Abstract
Background DNA repair gene mutations are present in 8–10% of localized prostate cancers. It is unknown whether this is influenced by clinicopathologic factors.

Methods We interrogated localized prostate adenocarcinomas with tumor DNA sequencing information from the TCGA validated (n = 333) and Nature Genetics (n = 377) datasets. Homologous recombination repair genes included in our analysis were: ATM, BRCA1/2, CDK12, CHEK1/2, FANCA, FANCD2, FANCL, GEN1, NBN, PALB2, RAD51, and RAD51C. Proportions of cases with pathogenic DNA repair mutations (and in ATM/BRCA1/2 specifically) were reported by Gleason grade group, clinical T, pathologic T, and pathologic N stage. Odds ratios and Fisher’s exact tests were used to compare proportions between categories.

Results Patients with Gleason grade groups 3 and higher were 2.2 times more likely to harbor any DNA repair mutation (95% CI: 1.2–4.2; 10.3% versus 5.0%) and were 2.7 times more likely to have BRCA1/2 or ATM mutations (95% CI: 1.3–6.6; 7.0% versus 2.7%) compared to those in Gleason grade groups 1–2. Patients with pathologic T3 and T4 stage (pT3/pT4) were 2.6 times more likely to have any DNA repair mutation (95% CI: 1.3–6.6; 13.0% versus 5.5%) and were 3.2 times more likely to have BRCA1/2 or ATM mutations (95% CI: 1.2–11.3; 9.5% versus 3.1%) compared to those with pT2 disease. There was no difference by clinical tumor or nodal stage. Among men with Gleason grade group ≥ 3 and clinical stage ≥ cT3, 21.3% (1 in 5) had a DNA repair mutation in any gene and 11.7% (1 in 9) had a mutation in ATM/BRCA1/2.

Conclusions The prevalence of pathogenic DNA repair gene alterations is enriched in men with advanced tumor stages and higher Gleason grade groups, with maximal enrichment observed in those with Gleason grade group ≥ 3 and clinical stage ≥ cT3 disease. This information can be used to guide eligibility criteria for genomically targeted clinical trials in the neoadjuvant/adjuvant settings.

Introduction

Approximately 160,000 patients will be diagnosed with prostate cancer annually [1]. While most of these men will be cured with surgery or radiation targeting the prostate alone, many will go on to develop disease recurrence. The natural history of prostate cancer can be quite variable and there have been many attempts to identify biomarkers and clinical features of the disease to accurately predict recurrence risk after local therapy with curative intent. The most widely accepted clinical features used to risk-stratify patients are tumor staging, nodal staging, and Gleason score [2–4].

With the development and incorporation of somatic next-generation DNA sequencing into clinical practice, there have been further attempts to use genomics to risk-stratify
patients and to select patients for targeted therapies. Within prostate cancer, genes in the DNA repair pathway have been found to be pathologically mutated in ~8–10% of localized prostate cancers [5, 6] and about 20–25% of metastatic castration-resistant prostate cancers [7]. BRCA1 and BRCA2 mutations, for example, have been associated with more aggressive forms of prostate cancer and with higher recurrence and mortality rates [8–11]. Furthermore, poly-ADP ribose polymerase (PARP) inhibitors have emerged as a potential therapeutic option for men harboring these mutations in the advanced prostate cancer setting [12, 13]. Identifying men harboring these mutations early could be helpful to guide therapies and clinical trial design, for example, when selecting patients for PARP inhibitor trials.

PARP inhibitors are currently being explored in multiple clinical trials in men with mutations in DNA repair pathway genes, particularly genes regulating the homologous recombination repair pathway (NCT03040791, NCT03442556, NCT03012321, NCT02952534, NCT02975934, NCT02854436). Given the lower prevalence of these mutations in localized prostate cancer compared to advanced disease, neoadjuvant and adjuvant trials targeting men with these mutations would be challenging to conduct if enrolling patients indiscriminately or if screening all patients for germline and somatic mutations (i.e., the number needed to screen (NNS) to find one patient with a homologous repair gene mutation would be very large). Histological variants, such as intraductal or ductal morphologies, and the presence of lymphovascular invasion, have both been shown to enrich for the presence of a germline DNA repair gene mutation [14]. It is currently unknown whether somatic mutations in DNA repair genes vary according to clinical or pathologic factors.

Therefore, we sought to determine if clinical factors used for risk-stratifying patients—i.e., Gleason score, tumor stage and nodal status—could also identify those men with prostate cancers more likely to harbor a DNA repair gene mutation, specifically a homologous repair mutation that may be used as an inclusion criterion for participation in a PARP inhibitor clinical trial. We hypothesized that such mutations would be more common in cases with higher Gleason score, higher tumor stage, and positive nodal status. We also sought to determine the best combination of clinicopathologic factors that would maximally enrich for the presence of DNA repair gene mutations, aiding in the selection of eligibility criteria for neoadjuvant/adjuvant clinical trials.

**Methods**

**Study design**

Genomic data was obtained from primary localized prostate adenocarcinoma cases from prostatectomies with somatic DNA sequencing data in The Cancer Genome Atlas (TCGA) repository validated dataset [6, 15, 16] and the Nature Genetics dataset [17] that are publically available on cBioPortal (http://www.cbioportal.org/). Gleason scores were available in both datasets, analyzed by Gleason sum, and also classified into their corresponding Gleason grade groups (1–5) to use a contemporary classification [18].

Clinical and pathologic tumor stage and pathologic nodal stage were available in the TCGA dataset only. Clinical tumor (cT) stage, pathologic tumor (pT) stage, and pathologic nodal (pN) stage were analyzed when available and were correlated with DNA repair gene mutation status. Clinical N stage and prostate-specific antigen (PSA) were excluded due to multiple missing data elements. Tumor stages T1 and T2 were combined as organ-confined disease and T3 and T4 were combined to represent locally invasive disease outside of the prostate, consistent with staging from the American Joint Committee on Cancer (AJCC) [19].

DNA repair gene mutations were defined as pathogenic alterations in the following genes: ATM, BRCA1/2, CDK12, CHEK1/2, FANCA, FANCD2, FANCL, GEN1, NBN, PALB2, RAD51, and RAD51C. These genes were selected based on the fact that they are involved in the homologous recombination repair pathway, and would thus be expected to enrich for sensitivity to PARP inhibitor therapy. A separate analysis was also conducted restricting the gene list to only BRCA1/2 and ATM specifically (since these three genes are anticipated to be most closely linked with PARP inhibitor response). For all genes, mutations were counted as pathogenic if they were homozygous gene deletions, protein-truncating DNA sequence alterations (frameshift mutations, nonsense mutations), or splice site mutations affecting the conserved splice acceptor or donor sites. Silent mutations were not included as pathogenic for the purposes of this analysis. From cBioPortal, it was not possible to distinguish mono-allelic from bi-allelic sequence alterations using this publicly available data, so both types of alterations were included.

**Statistical analysis**

The proportions of DNA repair gene mutations were reported as a whole and according to Gleason grade group, clinical T stage, pathologic T stage, and pathologic N stage. Odds ratios were calculated and the Fisher’s exact test was used to compare proportions between different groups. To ensure feasibility and interpretability we calculate sensitivity, specificity, population positive-predictive value, and population negative-predictive value for different cutoffs of clinical factors and different combinations of clinical factors. The population positive-predictive value and population negative-predictive value were calculated based on an estimate of a 10% prevalence of any DNA repair gene...
mutation and a 6% prevalence of \textit{BRCA1/2} and \textit{ATM} mutations specifically \cite{20}. The combination with the highest positive-predictive value was chosen as the optimal enrichment threshold. Analyses were performed using \textit{R} version 3.4.4 \cite{21}.

**Results**

**Overall prevalence of DNA repair gene mutations**

Within the entire population, 8.0\% of individuals (57 of 710) had any somatic DNA repair gene mutation, and 5.4\% had \textit{ATM} or \textit{BRCA1/2} mutations specifically. The five most commonly mutated genes were \textit{ATM} (2.7\%), \textit{BRCA2} (2.0\%), \textit{CDK12} (1.5\%), \textit{BRCA1} (0.7\%), and \textit{PALB2} (0.7\%) (Fig. 1). Mutation breakdown by dataset and clinical and pathologic factors are presented in the supplementary tables.

**Gleason grade group and DNA repair mutations**

There were a total of 657 evaluable patients (from both datasets) with Gleason score information. The prevalence of any DNA repair mutation was 4.5\% for Gleason grade group 1, 5.2\% in Gleason grade group 2, 10.5\% in Gleason grade group 3, 8.5\% in Gleason grade group 4, and 11.0\% in Gleason grade group 5 (Table 1, Fig. 2). Combined, those in Gleason grade groups 3 and higher had a prevalence of 10.3\% (number needed to screen (NNS) = 10) and were 2.2 times more likely (95\% CI: 1.2–4.2) to harbor a mutation compared to those in Gleason grade groups 1 and 2 (prevalence 5.0\%, (number needed to screen (NNS) = 20); \textit{p}-value for difference 0.01). Considering those who specifically had mutations in \textit{ATM} or \textit{BRCA1/2}, those with Gleason grade groups 3 and higher had a prevalence of 7.0\% (NNS = 15) and were 2.7 times more likely (95\% CI: 1.3–6.6) to have a mutation than those in Gleason grade groups 1 and 2 (prevalence 2.7\%, NNS = 37, \textit{p}-value for difference 0.01) (Table 1, Fig. 2).

**Tumor stage and DNA repair mutations**

There were 258 evaluable patients for clinical T stage. The prevalence of any DNA repair mutation was 4.7\% for cT1 stage, 10.7\% for cT2 disease, 15.8\% for cT3 and there were no mutated cases among those with cT4 disease. Of those patients with organ-confined disease by clinical exam (cT1 or cT2), 7.8\% were found to be mutation-positive (NNS = 13) compared to 15.0\% (NNS = 7) being mutation-positive if clinical stage was cT3 or cT4 (\textit{p} = 0.14) (Table 2). When only considering \textit{ATM} or \textit{BRCA1/2} mutations, 4.7\% of those with cT1 disease, 7.1\% of those with cT2 disease, 10.5\% with cT3 disease and no patients with cT4 disease were found to have pathogenic mutations in these genes (there were only 2 men with cT4 disease in our sample). There was no statistically significant difference between the prevalence of these mutations based on extent of organ involvement (6\% in cT1/T2 and 10\% in cT3/cT4; \textit{p} = 0.31) (Table 2).

There were 327 evaluable patients for pathologic T staging. Those who had pT2 disease had a 5.5\% prevalence of any DNA repair mutation, those with pT3 disease had a 12.9\% prevalence, and those with pT4 disease had a 16.7\% prevalence of any DNA repair mutation. Combined, those with pT3 or pT4 disease were more likely to have a DNA repair mutation (OR 2.6, 95\% CI: 1.1–6.6) and did have
significantly higher prevalence (13.0%; NNS = 8) compared to those with pT2 disease (5.5%; \(p = 0.03\); NNS = 19). Considering only ATM or BRCA1/2, those with pT3/ pT4 disease were 3.2 times as likely to have a DNA repair mutation (95% CI: 1.2–11.3) and had a significantly higher proportion of men harboring mutations in those genes (9.5%; NNS = 11) compared to cases with pT2 disease (3.1%; \(p = 0.04\); NNS = 33) (Table 2).

### Nodal stage and DNA repair mutations

There were 285 evaluable patients for pathologic N staging. Of those with no evidence of nodal disease (pN0), 10.7% had a mutation of any DNA repair gene (NNS = 10), which was not statistically significantly different from those with nodal disease (pN1) of whom 11.5% had a mutation in a DNA repair gene (\(p = 0.81\); NNS = 9). There was also no difference between the prevalence of mutations in ATM or BRCA1/2 specifically by nodal status (7.3% versus 7.7%; \(p = 1.0\); NNS = 14 versus 13) (Table 2).

### Combination strategies

Next, we sought to determine the best combination of clinical factors that would predict the highest prevalence of DNA repair gene mutations. Among men with Gleason grade group 3 and higher and clinical stage cT3 or cT4, 21.3% had any DNA repair gene mutation and 11.7% had a mutation in ATM or BRCA1/2. Using these criteria, this translates into having to screen 5 men to find one with any homologous repair mutation, or screening 9 men to find one with an ATM or BRCA1/2 mutation. Among men with Gleason grade group 3 and higher and pathologic stage pT3 or pT4, 14.2% had any DNA repair mutation and 9.7% had a mutation in ATM or BRCA1/2. Using these criteria, this translates into having to screen 7 men to find one with any homologous repair mutation, or screening 10 men to find one with an ATM or BRCA1/2 mutation. Among men who had Gleason grade group 3 and higher or clinical stage cT3/ cT4, 13.1% had at least one mutation in a DNA repair gene.

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**Table 1** Prevalence of DNA repair gene mutations according to Gleason grade group

| Gleason grade group | Any DNA repair mutation | ATM/BRA1/2 mutations |
|---------------------|-------------------------|----------------------|
|                     | Prevalence 95% CI N/Total Combined Prevalence | Prevalence 95% CI N/Total Combined Prevalence |
| 1                   | 4.5% 0.9–12.7 3/66 5.0% | 1.5% 0–8.2 1/66 2.7% |
| 2                   | 5.2% 2.7–8.8 12/233 10.3% | 3.0% 1.2–6.1 7/233 7.0% |
| 3                   | 10.5% 5.9–17.0 14/133 10.3% | 8.3% 4.2–14.3 11/133 7.0% |
| 4                   | 8.5% 3.2–17.5 6/71 5.6% | 6.5% 3.2–11.6 10/154 7.0% |
| 5                   | 11.0% 6.6–17.1 17/154 7.0% | 5.6% 3.2–11.6 10/154 7.0% |

\(p\)-value (trend) = 0.14

| NNS Number needed to screen, CI [in italics] Confidence interval |
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**Fig. 2** Bar graphs showing the prevalence of DNA repair mutation by Gleason grade group

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and 8.2% had a mutation in ATM or BRCA1/2 specifically. Using these criteria, this translates into having to screen 8 men to find one with any homologous repair mutation, or screening 13 men to find one with an ATM or BRCA1/2 mutation. Finally, among those who had Gleason grade group 3 and higher or pathologic stage pT3/pT4, 11.9% had at least one mutation in a DNA repair gene and 7.4% had a mutation in ATM or BRCA1/2 (Table 3). Using these criteria, this translates into having to screen 9 men to find one with any homologous repair mutation, or screening 14 men to find one with an ATM or BRCA1/2 mutation.

### Discussion

To our knowledge, this is the largest study to examine the prevalence of somatic alterations in DNA repair pathway genes (specifically, homologous recombination genes) in localized prostate cancer to help identify those who should be targeted for genomic screening. We report that mutations in DNA repair genes, and in ATM/BRCA1/2 specifically, are enriched in localized prostate cancers with more advanced Gleason scores (Gleason grade group ≥3, i.e., primary pattern 4 and higher). Additionally, we found that tumor extension outside of the prostate by pathologic stage was associated with a higher prevalence of DNA repair mutations. This is similar to what was observed in other studies of men harboring BRCA1 and BRCA2 germline mutations [8–11, 22]. In contrast to previous research, we did not find an association between the prevalence of DNA repair gene mutations and pathologic lymph node involvement. Lymph node metastases have been reported to be more common in men with germline BRCA1/2 mutations [9]. However, our research suggests this does not apply when considering somatic mutations in BRCA1/2 as well as other DNA repair gene mutations. This may be because there is a smaller difference and insufficient power to detect in our study, misclassification within the populations used, or confounding by other associations.

Currently, there is no consensus on which men with localized prostate cancer should undergo tumor mutational testing [23, 24]. Our primary motivation for conducting the present study was to inform clinical trial designs in the neoadjuvant and adjuvant spaces, particularly in the context of PARP inhibitor use. Taken together, the totality of our data suggest that the maximal enrichment for the presence of a somatic homologous recombination mutation occurs if patients are selected using a combination of Gleason grade group ≥3 histology (i.e., primary Gleason pattern 4 and above) and clinical stage T3 or T4 disease. Under these circumstances, 5 men would need to be screened to find one with any homologous repair mutation, and 9 men would need to be screened to find one with an ATM or BRCA1/2 mutation. Therefore, if, for example, one was designing a clinical trial testing a PARP inhibitor in the adjuvant setting in 50 patients with a homologous repair gene mutation, 235 such patients would need to be screened to find 50 eligible men. If the same trial targeted men with ATM/BRCA1/2 mutations more specifically, then 425 such patients would need to be screened to find 50 eligible subjects. This type of rational selection of patients based on particular Gleason grades and tumor stages would limit unnecessary screening of men who had a very low likelihood of harboring a DNA repair gene mutation and would greatly decrease the cost and timeline of such a study. If considering the general population of all men with localized prostate cancer who may benefit from genomic screening, we would need to screen 9 men to find one with an ATM or BRCA1/2 mutation, or 14 men to find one with any homologous repair mutation.

### Table 2

| Stage | Any DNA repair mutation | ATM/BRCA1/2 mutations |
|-------|-------------------------|----------------------|
|       | Prevalence  | 95% CI  | N/Total | Combined Prevalence | NNS | Prevalence  | 95% CI  | N/Total | Combined Prevalence | NNS |
| cT1   | 4.7%       | 1.5–10.7 | 5/106   | 7.8%       | 13  | 4.7%       | 1.5–10.7 | 5/106   | 6.0%       | 17  |
| cT2   | 10.7%      | 5.7–18.0 | 12/112  |           |     | 7.1%       | 3.1–13.6 | 8/112   |           |     |
| cT3   | 15.8%      | 6.0–31.3 | 6/38    | 15.0%      | 7   | 10.5%      | 2.9–24.8 | 4/38    | 10%       | 10  |
| cT4   | 0.0%       | 0–84.2   | 0/2     |           |     | 0.0%       | 0–84.2   | 0/2     |           |     |
|       | p-value (trend) = 0.14 | | | | | p-value (trend) = 0.08 | |
| pT2   | 5.5%       | 2.2–11.0 | 7/127   | 5.5%       | 19  | 3.1%       | 0.9–7.9  | 4/127   | 3.1%       | 33  |
| pT3   | 12.9%      | 8.5–18.4 | 25/194  | 13.0%      | 8   | 9.3%       | 5.6–14.3 | 18/194  | 9.5%       | 11  |
| pT4   | 16.7%      | 0.4–64.1 | 1/6     |           |     | 16.7%      | 0.4–64.1 | 1/6     |           |     |
|       | p-value (trend) = 0.05 | | | | | p-value (trend) = 0.46 | |
| pN0   | 10.7%      | 7.1–15.4 | 25/233  | 10.7%      | 10  | 7.3%       | 4.3–11.4 | 17/233  | 7.3%       | 14  |
| pN1   | 11.5%      | 4.4–23.4 | 6/52    | 11.5%      | 9   | 7.7%       | 2.1–18.5 | 4/52    | 7.7%       | 13  |
|       | p-value (trend) = 0.81 | | | | | p-value (trend) = 1.00 | |

NNS Number needed to screen
In conclusion, DNA repair gene mutations in localized prostate cancer are more prevalent in men with higher Gleason grades (Group 3 and higher) and more advanced clinical and pathologic stages (T3/T4 disease). When designing neoadjuvant/adjuvant clinical trials aimed at capturing homologous repair-deficient patients, these clinicopathologic characteristics can be used to determine eligibility criteria. Maximum enrichment for these PARP inhibitor-sensitivity mutations will occur in men with both Gleason grade group ≥ 3 and clinical stage ≥ cT3, where only 5 men would need to be screened to identify one with a homologous repair gene mutation (in any gene) and 9 men would need to be screened to find one with an ATM/BRCA1/2 mutation specifically. These findings may inform clinical trial design.

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Compliance with ethical standards

Conflict of interest Emmanuel S. Antonarakis is a paid consultant/advisor to Janssen, Astellas, Sanofi, Medivation, ESSA, AstraZeneca, Clovis and Merck; he has received research funding to his institution from Janssen, Johnson & Johnson, Sanofi, Genentech, Novartis, Tokai, Bristol Myers-Squibb, AstraZeneca, Clovis and Merck; and he is the co-inventor of a biomarker technology that has been licensed to Tokai and Qiagen. Catherine Handy Marshall received research support through the Conquer Cancer Foundation/Bristol Meyers Squibb. The remaining authors declare that they have no conflict of interest.

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