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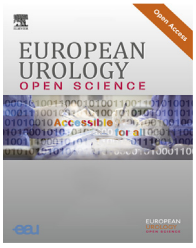

European Association of Urology

Prostate Cancer

Inherited Mutations in DNA Damage Repair Genes in Italian Men with Metastatic Prostate Cancer: Results from the Meet-URO 10 Study

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Abstract

Background: The prevalence of pathogenic germline mutations in DNA damage repair (gDDR) genes in the Italian population is unknown.
Objective: In this prospective multicenter cohort study, we evaluated the prevalence of gDDR alterations in the Italian population affected by metastatic prostate cancer (mPCa) and analyzed the impact on response to therapy, survival, and time to castration resistance.
Design, setting, and participants: In an observational prospective trial, 300 consecutive Italian mPCa patients, enrolled in the Meet-Uro-10 trial from three academic Italian centers, were recruited between 2017 and 2019 and were screened for gDDR mutations in 107 genes.
Outcome measurements and statistical analysis: The primary endpoint was to assess the prevalence of gDDR mutations in the Italian population of patients with mPCa. The secondary endpoints included the association of gDDR subgroups with metastatic onset, Gleason score, and time to castration resistance.
Results and limitations: We identified 297 valuable patients. Forty-six patients had a pathogenic/likely pathogenic variant (15.5%, 95% confidence interval: 11.4–19.6): the more frequent was *gBRCA2* found in nine cases (3%), followed by *gATM* in five cases (1.7%). In patients without mutations, longer median overall survival was observed with the sequence docetaxel-androgen receptor signaling inhibitor (ARSI) than with the sequence ARSI-docetaxel (87.9 vs 42 mo, $p = 0.0001$). In a univariate analysis, the median time to castration resistance in gDDR mutated patients

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was 19.8 mo, versus 23.7 mo in no mutated patients (p = 0.024). There were no associations of gDDR subgroups with metastatic onset and Gleason score ≥8. In our cohort, variants of unknown significance in gDDR genes were found in 80 patients and might have a prognostic relevance.

Conclusions: The study reported the prevalence of gDDR in the Italian population. The presence of *gBRCA2* mutations correlates with a shorter time to the onset of castration resistance disease.

Patient summary: The prevalence of *gBRCA2* in the Italian population is 3%, which is similar to that in the Spanish population, identifying similarities between people of the Western Mediterranean area.

1. Introduction

The oncological treatment of metastatic prostate cancer (mPCa) has changed dramatically in recent years, due to not only the development of novel drugs, but also the anticipation of various therapies from the metastatic castration-resistant prostate cancer (mCRPC) to the hormone-sensitive prostate cancer (PCa) setting. Nowadays, the challenge is represented by the identification of valid prognostic biomarkers of therapy response and the construction of a personalized therapy for each patient.

The increasing diffusion of genome-wide next-generation sequencing approaches has helped expand the knowledge about the heterogeneous molecular landscape of PCa [1–3].

As a consequence, the main therapeutically actionable molecular subtypes of PCa have been identified, in particular homologous recombination-deficient (HRD) [4], characterized by alterations in the homologous recombination repair (HRR) pathway and more strictly an HRD mutational signature [5,6], microsatellite instability-high (MSI-H), and CDK12-deficient tumors. DNA damage repair (DDR) genes are implicated in the mechanism of repair of DNA alterations during the cell cycle with the detection and repair of eventual DNA damage leading to programmed death of mutated cells. The presence of germline or somatic pathogenic variants of DDR genes reduces the ability to repair both single and double strand breaks of DNA damage. Notably, men with mPCa and DDR gene mutations have been reported to have sustained responses to poly-ADP ribose polymerase (PARP) inhibitors; recent clinical studies have demonstrated the efficacy of targeted treatment of specific molecular subtypes of mCRPC, that is, the aforementioned PARP inhibitors for HRD PCa, alone or in combination with an androgen receptor signaling inhibitor (ARSI) [7–11]. Moreover, testing patients for these genetic alterations has prognostic and predictive values for mCPRC patients [12].

Germline mutations of *BRCA1-2* are the most studied genes involved in the HRR system, and the presence of mutations correlates with an increased risk of developing breast, ovarian, prostatic, pancreatic, and colon cancers [13].

The incidence of germline pathogenic variants in DDR genes among patients with mPCa ranged between 11% and 33%, and *BRCA2* was the most frequent gene involved, with a prevalence of 5.3% [14]. However, these data refer to the Anglo-American population. The prevalence of *gBRCA2* in the Chinese population [15] was 4.3% and in the Spanish population 3.3% [16]. Furthermore, the prognostic value of non-BRCA2 DDR defects was less well defined.

This prospective study had the aim of evaluating the prevalence of germline mutations in DDR (gDDR) alterations in the Italian population affected by mPCa with the purpose to assess the genomic risk and provide an epidemiological profile of this population.

2. Patients and methods

This is an Italian multicenter observational prospective trial (Meet-Uro-10 trial). The primary endpoint was to estimate the prevalence of gDDR mutations in the Italian population of patients affected by mPCa.

The secondary endpoints were to analyze the impact of germline mutations in *BRCA2*, *ATM*, *PALB2*, and other gDDR genes on response to therapy, survival, and time to castration resistance.

Between June 2017 and December 2019, 300 consecutive Italian patients from three Italian institutions (Meldola, Padova, and Genova) with mPCa and unknown mutational status were recruited. They had to have histologically confirmed PCa and age ≥18 yr, to start a first-line treatment. The study was approved by each local ethical committee, and all patients provided informed consent at study entry.

At the baseline, a radiological imaging evaluation was required (computed tomography/magnetic resonance imaging, and bone scan) as well as a complete full blood count and biochemistry, including PSA and testosterone within 2 wk of study entry. Furthermore, a 5-ml blood sample was drawn at study entry for germline DNA extraction. Germline DNA extraction was performed using QIAamp DNA Mini kit (Qiagen) following the manufacturer's instructions. Germline variants analyses were performed in CNIO center in Spain, with the same workflow described in the aforementioned work [16].

This is a nonpharmacological, prospective study: any decision about drug administration is made by the physician based on his clinical judgment in the context of clinical practice, independently from the decision to include the patient in this study. Patients were evaluated through standard assessment according to clinical practice.

Responses and progression were evaluated according to the Prostate Cancer Clinical Trials Working Group 2 [17].

2.1. Statistical analysis

Descriptive statistics such as absolute frequencies and percentage were used for variables measured on a nominal or ordinal scale, and median values and interquartile ranges (IQRs) were used for variables measured on a continuous scale.

Comparisons of the median values of the markers within the different clinical characteristics were made using the nonparametric Wilcoxon test of medians, while comparisons of frequencies were made using the chi-square test.

Time to castration resistance was calculated as the time between androgen deprivation therapy initiation date and castration resistance onset date.

Progression-free survival (PFS) was calculated as the time between the date of treatment start and the date of disease progression for patients who had disease progression or the date of death for patients who died without evidence of disease or the date of the last tumor evaluation for patients who have not had disease progression.

Overall survival (OS) was calculated as the time from the date of onset of castration resistance to the date of death for deceased patients or the last follow-up for living patients.

The curves of the time-dependent variables were determined with the Kaplan-Meier limit product method, and the relative comparisons were performed according to the log-rank test.

All p values were obtained considering two-sided tests, and statistical analyses were performed with SAS statistical software, version 9.4 (SAS Institute, Cary, NC, USA).

3. Results

Between June 2017 and December 2019, 300 mPCa patients were enrolled. Despite multiple attempts, the germline DNA samples from three patients could not be analyzed.

At the time of PCa diagnosis, the median age of participants was 66 yr (IQR 61–72) and 106 (53.7%) patients already had metastatic disease (Table 1). Except for four, all patients were Caucasian with Italian ancestry. Of the evaluable cases, 172 (58%) were from the Emilia-Romagna region, 27 (9%) from the Marche region, 20 from the Veneto region (7%), and 78 (26%) from other Italian regions. Forty-six patients (15.5%, 95% confidence interval [CI]: 11.4–19.6) had a pathogenic/likely pathogenic variant (Fig. 1): the more frequent was *gBRCA2* in nine cases (3%), *gATM* in five (1.7%), *gMUTYH* in three cases (1%), *gCHEK2* in three cases (1%), and *PALB2* in one case (0.3%). No mutations in *BRCA1* were identified. We also highlighted five patients (1.7%) with clonal hematopoiesis of indeterminate potential interference [18] in ATM (n = 2), ATR (n = 1), CHEK1 (n = 1), and BRIP1 (n = 1). Finally, we identified 117 alterations of variants of unknown significance (VUSs) in 80 patients, having neutral/low clinical significance [19].

Therefore, we divided our cohort into three groups: patients with *gBRCA2* mutations (group A), patients with mutations in DDR genes other than *gBRCA2* (group B), and patients without mutations (group C). In detail, group B included the following genes: *ATM*, *BLM*, *BRCA1*, *BRIP1*, *CHEK2*, *FAN1*, *FANCA*, *FANCC*, *FANCD2*, *FANCI*, *MLH1*, *MSH2*, *MUTYH*, *NBN*, *PALB2*, *PMS2*, *POLE 2*, and *RAD54L*.

In a univariate analysis, the median time to castration resistance in group A + B was 19.8 mo (95% CI: 10.9–23.9) versus 23.7 mo (95% CI: 19.7–26.4) in group C (p = 0.02; Table 2 and Fig. 2). In particular, patients with *gBRCA2* mutations experienced the worse time to castration resistance with a median of 11.6 mo (95% CI: 3.3–21.2). There was no association with *gBRCA2* mutations and metastatic onset, nor with Gleason score (adopting a threshold of $</\geq 8$), and the same was observed for patients with other gDDR mutations. In a multivariate analysis, gDDR mutation was an independent prognostic factor for a shorter time to castration resistance, when adjusted for age, Gleason score (adopting a threshold of $</\geq 8$), lines of therapies (adopting a threshold of $</\geq 3$), and metastatic onset (hazard ratio [HR]: 1.58; 95% CI: 1.08–2.32; p = 0.02).

In the univariate analysis, patients of group A + B had longer PFS with the sequence docetaxel-ARSI (33.1 mo; 95% CI: 15.0–78.0) than with the sequence ARSI-docetaxel (16.8 mo; 95% CI: 8.3–22.8; p = 0.04). Furthermore, also in patients of group C, the median PFS was longer with the sequence docetaxel-ARSI (31.5 mo; 95% CI: 22.6–58.7) than with the sequence ARSI-docetaxel (21.1 mo; 95% CI: 18.0–24.0; p = 0.0001; Table 3).

Median OS of 87.9 mo (95% CI: 52.0–107.1) was observed in patients of group C treated with the sequence docetaxel-ARSI versus 42 mo (95% CI: 30.9–45.9) for ARSI-docetaxel (p = 0.0001). It was interesting to report that the median OS of patients belonging to group C, with the exclusion of patients with VUSs, was 111.4 mo (95% CI: 52.0–not reached) with the sequence docetaxel-ARSI versus 41.3 mo (95% CI: 29.7–52.6) in patients treated with ARSI-docetaxel (p = 0.0003). In the univariate analysis, in group A + B, no significant difference in median OS was observed according to the sequence of treatment (Table 3). In the multivariate analysis, the sequence of treatment was an independent prognostic factor (Table 4).

4. Discussion

To our knowledge, Meet-Uro-10 is the first prospective non-interventional trial that investigates the prevalence and impact of gDDR mutations in the Italian population affected by mPCa. Since it is a prospective cohort of patients, we acknowledge a potentially lower impact of a selection bias that usually belongs to retrospective and interventional studies [20].

Several studies reported the prevalence of gDDR mutations in PCa in different populations. Nicolosi et al [21] described a large series of PCa patients (n = 3607), observing the highest rates of gDDR alterations in patients of Ashkenazi Jewish descent (22.7%), followed by Caucasian (17.8%), Asian (15.1%), African American (10.1%), and Hispanic (6.4%) men. The prevalence of gDDR mutations in different populations is shown in Table 5.

Concerning single genes, it is interesting that the prevalence of gBRCA2 mutations in our cohort is lower than that reported in the USA + UK and Chinese populations, but similar to that in the Spanish population [13,15,16]. No gBRCA1 mutation was found in our cohort; we explain this as a stochastic result related to the low prevalence of this mutation rather than the absence of gBRCA1 in the Italian population. In our work, no correlation was made with somatic mutations, but it is difficult to infer the prevalence of

Table 1 – Patient characteristics (*n* = 297)

Characteristics	gBRCA2 patients (*n* = 9)	Other mutations (*n* = 37)	Alterations of VUS (*n* = 80)	No mutated patients (*n* = 171)	*p* value
Age at diagnosis, median (IQR)	66 (59–71)	65 (59–69)	65 (60–70)	67 (61–73)	0.5
Age at time to castration resistance, median (IQR)	71 (61–71)	71 (65–78)	72 (64–77)	73 (67–79)	0.1
Gleason score, *N* (%)					
≥8	6 (75.0)	21 (65.6	46 (71.9)	87 (59.6)	
<8	2 (25.0)	11 (34.4)	18 (28.1)	59 (40.4)	0.3
Unknown/missing	1	5	16	25	
Stage at diagnosis, *N* (%)					
M0	5 (55.6)	25 (67.7)	50 (62.5)	104 (60.8)	
M1	3 (44.4)	11 (32.3)	30 (37.5)	62 (39.2)	0.8
Unknown/missing	1	1	0	5	
Site of metastases, *N* (%)					
M0	5	25	50	104	
Bone	1	5	16	38	
Lymph nodes	1	2	3	5	
Bone and lymph nodes	1	4	10	17	
Bone and lymph nodes and visceral disease	0	0	1	2	-
Unknown/missing	1	1	0	5	
Prostatectomy, *N* (%)					
No	4 (44.4)	21 (56.8)	40 (50.0)	101 (59.1)	
Yes	5 (55.6)	16 (43.2)	40 (50.0)	70 (40.9)	0.5
Radiotherapy, *N* (%)					
No	9 (100)	28 (75.7)	70 (87.5)	146 (85.4)	
Yes	0	9 (24.3)	10 (12.5)	25 (14.6)	0.2
Lines of therapies, *N* (%)					
≥3	2 (22.2)	14 (37.8)	34 (42.5)	55 (32.2)	
<3	7 (77.8)	23 (62.2)	46 (57.5)	116 (67.8)	0.3
Second tumor	1	6	9	18	–

IQR = interquartile range; VUS = variant of unknown significance.

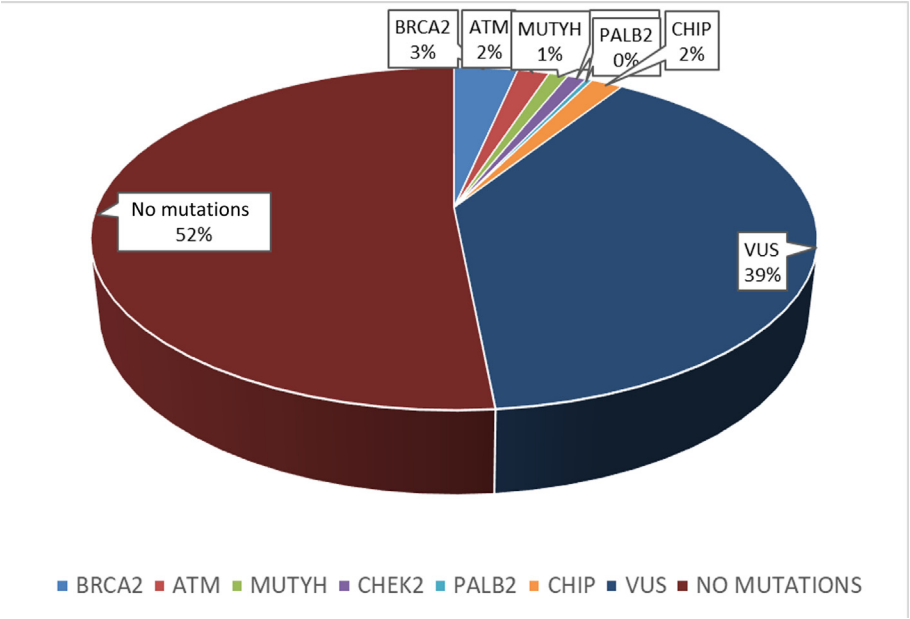

Fig. 1 – Inherited mutations in gDDR. gDDR = germline mutations in DNA damage repair; VUS = variant of unknown significance.

Table 2 – Univariate analysis of time to castration resistance (*n* = 280)

	No. of patients	No. of events	Time to castration resistance (mo),median (95% CI)	*p* value
DDR mutation (gBRCA2 mut included)	36	36	19.8 (10.9–23.9)	
Not mutated	244	244	23.7 (19.7–26.4)	0.02

CI = confidence interval; DDR = DNA damage repair; mut = mutation.

somatic mutations based on the percentage of germline mutations that we found in our cohort: a systematic review of the prevalence of DNA damage response gene mutations in PCa patients has highlighted differences based on each DDR gene analyzed; however, the authors underline the role of biases potentially harming their results [22]. Con-

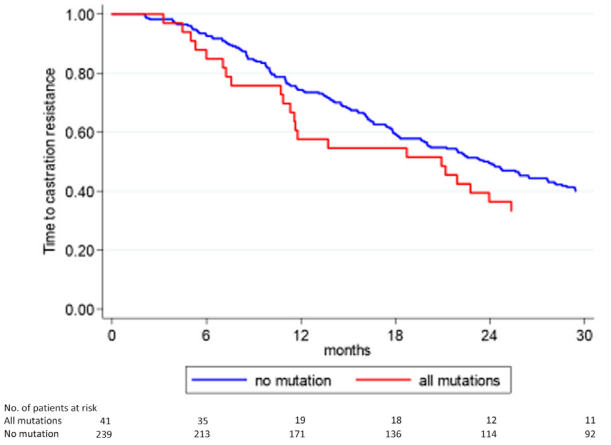

Fig. 2 – Median time to castration resistance in gDDR mutated versus nonmutated patients. gDDR = germline mutations in DNA damage repair.

cerning response to PARP inhibitors, there seems to be no difference between patients harboring germline and somatic BRCA mutations [9,23].

The different distribution of gDDR mutations highlighted the need of developing population-specific multiple gene panels for men with mPCa. In fact, the various types of gDDR variants may cause heterogeneous tumor biology and, ultimately, different responses to the same treatment among different populations.

In a retrospective study of 319 patients with mPCa, Annala et al [24] demonstrated that patients with DDR mutations treated with ARSIs as first-line treatment had significantly shorter PFS than noncarriers (3.3 vs 6.2 mo, p = 0.01). On the contrary, Antonarakis and colleagues [25], analyzing 172 mPCa patients, showed that men with mutations of *ATM* or *BRCA1/2* genes who received ARSIs as first-line treatment, experienced longer PFS with respect to noncarriers (15 vs 10.8 mo, p = 0.09). Prorepair-B is a prospective Spanish study that failed to demonstrate significant difference in cause-specific survival (CSS) between patients with DDR mutations and those without mutations (23.3 vs 33.2 mo; p = 0.264; HR: 1–32: 95% CI: 0.81–2.17); however, Castro and colleagues [16] confirmed that *gBRCA* mutations were an independent prognostic factor that correlates with shorter CSS (17.4 mo in men with *gBRCA2* mutations vs 33.2 mo in noncarriers; p = 0.027; HR: 2.10; 95% CI: 1.07–4.10). In a subgroup analysis, alterations of *BRCA2* correlated with a shorter CSS rate in mCRPC patients treated with the sequence docetaxel-ARSI (10.7 mo) than in those treated with the sequence ARSI-docetaxel (24.0 mo). The conflicting survival results about sequencing docetaxel-ARSI and vice versa—as reported in our current work and in the Prorepair-B trial—are difficult to explain to date and certainly needs further studies; however, these data suggest that the choice of that treatment sequence may be crucial for patients with *gBRCA2* mutations. It must be noted that the current therapeutic paradigm is changing due to new data regarding the association of chemotherapy and ARSIs in the first-line hormone-sensitive setting: in detail, the phase 3 trial ARASENS have demonstrated survival improvement by adding darolutamide to docetaxel in treatment-naïve patients [26]; prospective data on gDDR patients are needed to assess the impact of this new therapeutic approach.

Patients with *gBRCA2* mutations experienced a lower time from diagnosis to the development of castration resistance disease, confirming the association with a more aggressive phenotype and suggesting a potential high impact of PARP inhibitors in metastatic hormone-sensitive prostate cancer (mHSPC) [27]. In addition, our study also reported data about the impact of gDDR aberrations on the outcomes of patients with mCRPC; in particular, we highlighted that patients without mutations had better outcomes when treated with taxanes as first-line therapy. This benefit seems greater if we excluded VUSs from the analysis, suggesting that VUSs might be a prognostic factor.

In this contest, it is important to highlight limitations in studying VUSs, in particular, the challenge of differentiating pathogenic from nonpathogenic VUSs. It has been reported that up to 20% of BRCA sequencing results are VUSs, more than half of which are missense mutations [28].

In a recent study by Darst and colleagues [29], VUSs appeared to have no association with aggressive PCa for the large majority of the genes assessed. However, they presented some evidence that VUSs in a few rarely altered genes are linked with aggressive PCa and/or mPCa.

Table 3 – Univariate analysis of overall survival (OS) and progression-free survival (PFS) in patients treated with different sequences of docetaxel and ARSI as first and/or second line of therapy

	No. of patients	No. of deaths	OS (mo), median (95% CI)	p value	No. of progressions	Median PFS (mo), median (95% CI)	p value
DDR mutation (gBRCA2mut included)							
Docetaxel→ARSI	7	5	79.8 (26.5–NR)		7	33.1 (15.0–78.0)	
ARSI→docetaxel	8	5	69.2 (20.7–NR)	0.1	8	16.8 (8.3–22.8)	0.04
BRCA2 mutation							
Docetaxel→ARSI	1	0	49.6 (–)		1	33.1 (–)	
ARSI→docetaxel	2	2	25.5 (20.7–NR)	0.2	2	12.3 (8.3–NR)	0.2
Not mutated							
Docetaxel→ARSI	31	21	87.9 (52.0–107.1)		28	31.5 (22.6–58.7)	
ARSI→docetaxel	73	54	42.0 (30.9–45.9)	0.0001	71	21.1 (18.0–24.0)	0.0001
Not mutated (VUS excluded)							
Docetaxel→ARSI	24	10	111.4 (52.0–NR)		19	34.5 (22.2–87.2)	
ARSI→docetaxel	51	36	41.3 (29.7–52.6)	0.0003	49	19.0 (16.4–26.5)	0.0002

ARSI = androgen receptor signaling inhibitor; DDR = DNA damage repair; NR = not reached; VUS = variant of unknown significance.

Table 4 – Multivariate analysis

	Time to castration resistance		PFS		OS	
	HR (95% CI)	*p* value	HR (95% CI)	*p* value	HR (95% CI)	*p* value
Age (continuous variable)	0.985 (0.969–1.001)	0.06	1.020 (0.991–1.050)	0.1	1.036 (0.994–1.079)	0.09
Gleason score						
≥8	1.20 (0.91–1.57)	0.2	0.93 (0.61–1.42)	0.7	1.29 (0.79–2.12)	0.3
<8	1.00		1.00		1.00	
Stage at diagnosis						
M0	1.00		1.00		1.00	
M1	2.00 (1.49–2.69)	<0.0001	1.52 (0.99–2.33)	0.057	2.04 (1.25–3.33)	0.004
Lines of therapies						
≥3	1.45 (1.10–1.93)	0.009	1.33 (0.83–2.13)	0.2	0.54 (0.32–0.91)	0.02
<3	1.00		1.00		1.00	
Mutation status						
DDR mutation (gBRCA2 mut included)	1.58 (1.08–2.32)	0.02	1.41 (0.79–2.53)	0.2	1.06 (0.51–2.20)	0.9
Not mutated	1.00		1.00		1.00	
Sequence of treatment						
Docetaxel→ARSI	–		1.00		1.00	
ARSI→docetaxel	–		2.98 (1.82–4.89)	<0.0001	3.52 (1.96–6.33)	<0.0001

ARSI = androgen receptor signaling inhibitor; CI = confidence interval; DDR = DNA damage repair; HR = hazard ratio; OS = overall survival; PFS = progression-free survival.

Table 5 – Distribution of DDR mutations in different ethnic population

	Our cohort	USA + UK (Pritchard 2016 [13])	Spanish (Castro 2019 [16])	African American (Sartor 2022) [35]	Chinese (Zhu 2022 [15])
Gene	*N* = 297	*N* = 692	*N* = 419	Up to 214	Up to 1836
ATM	1.68	1.59	1.91	0.97	1.04
ATR	NA	0.30	0	NA	0.29
BLM	1.01	NA	NA	NA	NA
BRCA1	0	0.90	0.95	1.41	0.21
BRCA2	3.03	5.30	3.30	2.80	4.30
BRIP1	0.67	0.14	0	0	0.06
CHEK2	1.01	1.40	0.50	0.48	0.17
FAN1	0.67	NA	NA	NA	NA
FANCA	0.34	NA	0	NA	0.30
FANCC	0.34	NA	0	NA	NA
FANCD2	0.34	NA	0.72	NA	NA
FANCI	0.34	NA	0	NA	NA
MLH1	0.34	0	0	0	NA
MSH2	0.34	0.14	2.00	0	0.45
MSH6	NA	NA	0	0	0.17
MUTYH	1.01	NA	3.10	NA	NA
NBN	0.34	0.30	0	0	0.06
PALB2	0.34	0.40	0	1.10	0.67
PMS2	0.67	0.30	0	0.47	0.06
POLE 2	0.67	NA	NA	NA	NA
RAD51C	NA	0.14	0	0.68	0.06
RAD51D	NA	0.40	0	0	0.25
RAD54L	0.34	NA	0.24	NA	NA

DDR = DNA damage repair; NA = not available.

VUSs are DNA alterations without strong evidence to be classified as either pathogenic or nonpathogenic; however, there is a small possibility that VUSs will be reclassified and their role will be reconsidered as new knowledge emerges. Therefore, an improvement in classification of VUSs is needed to investigate this form of coding variation, particularly for genes with evidence of pathogenetic variants associated with aggressive PCa.

Furthermore, in our cohort, *gBRCA2* mutated patients experienced more prolonged PFS when treated with docetaxel as first-line therapy followed by an ARSI as second-line therapy, rather than the reverse sequence. In literature, we have contradictory results about this topic. In detail, Castro and colleagues [16] observed that *gBRCA2* mutated patients benefit from receiving ARSIs as first-line treatment, while Antonarakis and colleagues [25] reported a trend toward longer PFS in both gDDR carriers and *ATM/BRCA1/BRCA2* carriers treated with ARSIs, compared with noncarriers (13.3 vs 10.3 mo, $p = 0.107$, and 15 vs 10.8 mo, $p = 0.09$, respectively). Annala and colleagues [24] reported contradictory results: they found significantly shorter PFS of gDDR carriers on first-line ARSIs, compared with noncarriers (3.3 vs 6.2 mo; $p = 0.01$).

In the near future, new scores to help clinicians in the identification of candidate patients for genetic counseling are needed, and a large international pooled analysis of germline data would be necessary to define new scores, considering differences among geographic regions.

We acknowledge several limitations to this study. First, the distribution and prevalence of gDDR mutations were extrapolated by a nonhomogeneous Italian population and may not be generalized for the whole population. An imple-

mentation of the screening is requested in order to establish the true frequency of inherited mutations in this subset of patients. Second, the analysis of response to treatment type and sequence is limited by the small number of carriers treated with each drug, by the lack of randomization, and by the physician-led therapeutic approaches, which introduces a potential bias in treatment choices. Third, this study did not investigate somatic DDR alterations, which could influence response to systemic treatment and, as a consequence, prognosis.

Therefore, findings about the impact of treatment sequence in *BRCA2* carriers and in patients without mutations should be considered hypothesis generating only until validated in larger series. The presence of gBRCA2 mutations and/or other gDDR mutations should also be correlated with other biomarkers with diagnostic and prognostic implications in PCa [30–34].

In conclusion, these results reinforce the need for genomic testing earlier in the clinical history for patients with mHSPC, to identify those harboring HRR gene-altered cancers especially BRCA1/2, because of their clinical impact and hereditary/familial cancer implications.

5. Conclusions

This study confirms that *gBRCA2* is the most common alteration in DDR genes in unselected patients with mCRPC. The prevalence of *gBRCA2* mutations in the Italian population is 3.4%, which is similar to that of the Spanish and Afro-American populations, and lower than that of the Chinese and Anglo-American populations, highlighting similarities among people of Western Mediterranean area and Afro-Americans. The presence of gDDR mutations and, in particular, the presence of *gBRCA2* mutations correlate with lower time to the onset of castration resistance disease, according to a more aggressive phenotype, which could represent significant information for the studies investigating PARP inhibitors in the mHSPC scenario. Patients without gDDR mutations experienced a better outcome when treated with the sequence docetaxel-ARSI than when treated with ARSI-docetaxel, suggesting a potential role as a predictive biomarker that needs other prospective confirming trials.

***Author contributions*:** Ugo De Giorgi had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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