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Germline Mutations in *ATM* and *BRCA1/2* Are Associated with Grade Reclassification in Men on Active Surveillance for Prostate Cancer

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Abstract

Background: Mutations in DNA repair genes are associated with aggressive prostate cancer (PCa).
Objective: To assess whether germline mutations are associated with grade reclassification (GR) in patients undergoing active surveillance (AS).
Design, setting, and participants: Two independent cohorts of PCa patients undergoing AS; 882 and 329 patients from Johns Hopkins and North Shore, respectively.
Outcome measurements and statistical analysis: Germline DNA was sequenced for DNA repair genes, including *BRCA1/2* and *ATM* (three-gene panel). Pathogenicity of mutations was defined according to the American College of Medical Genetics guidelines. Association of mutation carrier status and GR was evaluated by a competing risk analysis.
Results and limitations: Of 1211, 289 patients experienced GR; 11 of 26 with mutations in a three-gene panel and 278 of 1185 noncarriers; adjusted hazard ratio (HR) = 1.96 (95% confidence interval [CI] = 1.004–3.84, $p = 0.04$). Reclassification occurred in six of 11 carriers of *BRCA2* mutations and 283 of 1200 noncarriers; adjusted HR = 2.74 (95% CI = 1.26–5.96, $p = 0.01$). The carrier rates of pathogenic mutations in the three-gene panel, and *BRCA2* alone, were significantly higher in those reclassified (3.8% and 2.1%, respectively) than in those not reclassified (1.6% and 0.5%, respectively; $p = 0.04$ and 0.03, respectively). Carrier rates for *BRCA2* were greater for those reclassified from Gleason score (GS) 3 + 3 at diagnosis to GS $\geq$4 + 3 (4.1% vs 0.7%, $p = 0.01$) versus GS 3 + 4 (2.1% vs 0.6%; $p = 0.03$). Results are limited by the small number of mutation carriers and an intermediate end point.
Conclusions: Mutation status of *BRCA1/2* and *ATM* is associated with GR among men undergoing AS.
Patient summary: Men on active surveillance with inherited mutations in *BRCA1/2* and *ATM* are more likely to harbor aggressive prostate cancer.

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1. Introduction

Active surveillance (AS) is the recommended management strategy for men with favorable-risk prostate cancer (PCa) [1,2]. While cancer-specific outcomes are comparable for AS and curative intervention, some men left untreated develop disease progression [3,4].

Older age [5]and African American race [6] are associated with reclassification to a higher-grade cancer among men on AS. Family history does not appear to predispose to disease progression on AS [7]. Germline mutations contribute to familial disease; however, the association between changes in cancer grade and germline mutations in AS is not known.

Mutations in DNA repair genes are associated with aggressive PCa phenotypes [8,9]. A comparison between men with lethal and men with low-risk PCa found that mutation carrier rates of *BRCA1/2* and *ATM* were significantly higher in lethal cases [10]. Furthermore, carrier rates were directly and significantly associated with earlier age at death and shorter survival after diagnosis.

To our knowledge, germline mutations in PCa-associated genes have not been evaluated among men undergoing AS. We hypothesized that mutation status and grade reclassification (GR) to a higher grade during AS might be associated.

2. Materials and methods

2.1. Study cohorts

AS was offered to patients as part of an institutional review board-approved protocol at Johns Hopkins (JH) [5] and North Shore (NS) University Health Systems [11]. The JH program was initiated in 1995 and NS in 2011. After enrollment, a confirmatory biopsy was performed at 6–12 mo followed by prostate-specific antigen (PSA) and digital rectal examination at 6-mo intervals. Surveillance biopsies are recommended every 2–4 yr after confirmation biopsy.

2.1.1. JH

Since inception in 1995 to analysis in February 2018, 1764 men with favorable-risk PCa enrolled in AS. Median follow-up after enrollment was 4.0 (range, 0.01–20.0) yr. The median interval between surveillance biopsies was 1.0 (range, 0.3–11) yr.

2.1.2. NS

From 2011 to analysis in February 2018, 467 men with favorable- and intermediate-risk PCa enrolled in AS. Median follow-up after enrollment was 3 (range, 0.01–8.5) yr. The median interval between surveillance biopsies was 2 (range, 0.3–4.5) yr.

2.1.3. Final study cohort

Of 1764 men enrolled at JH, 872 men had no blood sample available for DNA extraction and 10 had missing data, leaving 882 for germline sequencing. Of 467 men enrolled at NS, 101 had no blood sample and 37 had missing data, leaving 329 men for germline sequencing. Thus, the final study cohort for gene sequencing was 1211 men; 882 and 329 patients from JH and NS cohorts, respectively (Supplementary Fig. 1).

2.2. Germline sequencing

Germline DNA from each patient was sequenced for *BRCA1/2* and *ATM* (three-gene panel) as a primary objective based on the evidence that these three genes are most relevant with respect to an aggressive phenotype (lethal/metastatic disease) [8,10,12,13], and for 51 DNA repair

Table 1 – Demographic descriptions

	Combined cohort (n = 1211)	Johns Hopkins AS cohort (n = 882)	North Shore AS cohort (n = 329)	p value [a]
Characteristic at diagnosis				
Age, median (IQR)	66 (61–70)	66 (61–69)	66 (61–70)	0.8
PSA, median (IQR)	4.70 (3.43–6.20)	4.80 (3.50–6.20)	4.55 (3.36–5.86)	0.2
PSA density, median (IQR)	0.10 (0.07–0.14)	0.10 (0.07–0.14)	0.10 (0.06–0.16)	0.9
No. of positive cores, median (IQR)	1 (1–2)	1 (1–2)	1 (1–2)	<0.001
Race, n (%)				
Caucasian	1119 (92.54)	796 (90.3)	323 (98.2)	<0.001
African American	60 (5.0)	57 (6.4)	3 (0.9)	
Others	32 (2.6)	29 (3.3)	3 (0.9)	
Prognostic GG at diagnosis, n (%)				
GG1	1182 (97.6)	878 (99.6)	304 (92.4)	<0.001
GG2	26 (2.2)	4 (0.4)	22 (6.7)	
GG3	3 (0.2)	0 (0)	3 (0.9)	
>2 cores positive, n (%)	141 (12)	63 (7)	78 (24)	<0.001
Follow-up characteristics [b]				
Follow-up in men at risk, yr, median (IQR)	4 (2–6)	5 (3–7)	3 (1–4)	<0.001
Time from diagnosis to confirmatory biopsy in years, median (IQR)	1 (1–1)	1 (1–1)	1 (0.5–1)	0.4
No. favorable (non-GG >1) surveillance biopsies, median (IQR)	2 (1–4)	3 (1–5)	1 (1–2)	<0.001

AS = active surveillance; GG = grade group (1 = Gleason score 3 + 3; 2 = Gleason score 3 + 4; 3 = Gleason score 4 + 3); IQR = interquartile range; PSA = prostate-specific antigen.

[a] Comparison between Johns Hopkins and North Shore AS cohorts (t tests and Mann-Whitney tests were used for continuous variables and Fisher's exact test for categorical variables). Note that the number of positive cores was significantly different between Johns Hopkins and North Shore despite similar median and IQR because the shape of the distributions differed significantly.

[b] Among 882 men from Johns Hopkins, there were a total of 4036 biopsies, including 882 confirmatory biopsies (8% MRI-guided) and 2272 surveillance biopsies (10% MRI-guided). In the North Shore cohort, there were a total of 1097 biopsies comprising 343 confirmatory (21% MRI-guided) and 339 surveillance biopsies (28% MRI-guided).

genes (Supplementary Table 1) as a secondary objective, using a targeted next-generation sequencing panel at NS University Health System Genomics Core Laboratory. All the targeted bases in these genes were successfully sequenced (>20 ×) in >99% samples.

2.3. Bioinformatics analysis

Paired-end reads were aligned to the GRCh37 version of the human genome using Burrows-Wheeler Aligner version 0.7 to generate BAM files [14]. After sorting the BAM files using samtools, polymerase chain reaction duplicates marked using Picard and realignment around putative gaps was performed using the Genome Analysis Toolkit (GATK) version 3.2-2. Variant calling was performed with the GATK Haplotype caller. ANNOVAR (http://annovar.openbioinformatics.org/en/latest) and snpEff were used for annotating variants and for retrieving information on variants in the population-based studies such as the 1000 Genomes Project (www.1000genomes.org), NHLBI-ESP 6500 exomes or ExAC (http://exac.broadinstitute.org/), and clinical databases such as the Human Gene Mutation Database [15] and ClinVar [16]. Pathogenicity of variants was defined based on American College of Medical Genetics and Genomics criteria [17]. Specifically, pathogenic and likely pathogenic mutations were defined as: (1) all protein truncating mutations unless their allele frequency was ≥5% in any racial group in population databases or was reported as benign or likely benign in the ClinVar, (2) non-synonymous changes if their allele frequency was <5% and reported as pathogenic and likely pathogenic mutations in the ClinVar, and (3) inframe-shift mutations affecting more than three amino acids were considered pathogenic mutations.

2.4. Statistical analysis

The frequency of pathogenic and likely pathogenic mutations was estimated for each gene. Our primary outcome of interest was GR, defined as an increase in Gleason score (grade group [GG]) on a biopsy after diagnosis, as a function of mutation carrier status. The association of mutation carrier status and GR was evaluated using a competing risk analysis [18,19] that accounted for elective treatment in the absence of reclassification, volume reclassification, and death due to causes other than PCa. Time zero was the time of diagnosis, and time to event was calculated between diagnosis and time of reclassification. Active men who did not reclassify and those lost to follow-up were censored at the time of last biopsy. The multivariable analysis was adjusted for the covariates of age, PSA density, the number of biopsy cores with cancer, and first two eigens (principal components) of genetic background. Eigen values were estimated from a principal component analysis of all variants in the sequenced data using the program ADMIXTURE (version 1.3) [20].

Hazard ratio (HR) and 95% confidence interval (CI) were calculated to estimate reclassification risk. Subset analyses were performed to evaluate outcomes specific for JH and NS, and GR from GG 1 (Gleason score 3 + 3) at diagnosis to 2 (Gleason score 3 + 4), and GG 1 at diagnosis to ≥3 (Gleason score ≥4 + 3). A type I error of 0.05 (two-sided) was used to define statistical significance, and analyses were performed using

Table 2 – Carrier rates of mutations in men with and without upgrading

Any upgrading on any biopsy after diagnostic biopsy									
	All (*n* = 1211)			Johns Hopkins (*n* = 882)			North Shore (*n* = 329)		
Gene	Upgraded (*n* = 289)	Non-upgraded (*n* = 922)	*p* value [a]	Upgraded (*n* =201)	Non-upgraded (*n* = 681)	*p* value [a]	Upgraded (*n* =88)	Non-upgraded (*n* = 241)	*p* value [a]
BRCA1, *n* (%)	4 (1.38)	7 (0.76)	0.30	1 (0.50)	5 (0.73)	>0.99	3 (3.41)	2 (0.83)	0.1
BRCA2, *n* (%)	6 (2.08)	5 (0.54)	0.03	3 (1.49)	4 (0.59)	0.20	3 (3.41)	1 (0.41)	0.06
ATM, *n* (%)	1 (0.35)	4 (0.43)	>0.99	1 (0.50)	2 (0.29)	0.54	0	2 (0.83)	>0.99
All [b], *n* (%)	11 (3.81)	15 (1.63)	0.04	5 (2.49)	11 (1.62)	0.38	6 (6.82)	4 (1.66)	0.03
Upgrading from GG1 to ≥3 [c]									
	All (*n* = 1182)			Johns Hopkins (*n* = 878)			North Shore (*n* = 304)		
Gene	Upgraded (*n* = 98)	Non-upgraded (*n* = 1084)	*p* value [a]	Upgraded (*n* =66)	Non-upgraded (*n* = 812)	*p* value [a]	Upgraded (*n* =32)	Non-upgraded (*n* = 272)	*p* value [a]
BRCA1, *n* (%)	1 (1.02)	9 (0.83)	0.58	0	6 (0.74)	>0.99	1 (3.13)	3 (1.10)	0.4
BRCA2, *n* (%)	4 (4.08)	7 (0.65)	0.01	2 (3.03)	5 (0.62)	0.09	2 (6.25)	2 (0.74)	0.06
ATM, *n* (%)	0	5 (0.46)	>0.99	0	3 (0.37)	>0.99	0	2 (0.74)	>0.99
All [b], *n* (%)	5 (5.10)	20 (1.85)	0.049	2 (3.03)	14 (1.72)	0.34	3 (9.38)	6 (2.21)	0.06
Upgrading from GG 1 to ≥2 [c]									
	All (*n* = 1182)			Johns Hopkins (*n* = 878)			North Shore (*n* = 304)		
Gene	Upgraded (*n* = 283)	Non-upgraded (*n* = 899)	*p* value [a]	Upgraded (*n* =200)	Non-upgraded (*n* = 678)	*p* value [a]	Upgraded (*n* =83)	Non-upgraded (*n* = 221)	*p* value [a]
BRCA1, *n* (%)	4 (1.41)	6 (0.67)	0.26	1 (0.50)	5 (0.74)	>0.99	3 (3.61)	1 (0.45)	0.06
BRCA2, *n* (%)	6 (2.12)	5 (0.56)	0.03	3 (1.50)	4 (0.59)	0.20	3 (3.61)	1 (0.45)	0.06
ATM, *n* (%)	1 (0.35)	4 (0.45)	>0.99	1 (0.50)	2 (0.29)	0.54	0	2 (0.90)	>0.99
All [b], *n* (%)	11 (3.89)	14 (1.56)	0.03	5 (2.50)	11 (1.62)	0.38	6 (7.23)	3 (1.36)	0.01

GG = grade group.
[a] Comparison of the proportion of men upgraded and not upgraded (Fisher's exact test).
[b] Any of three gene mutations (*BRCA1/2* or *ATM*).
[c] Grade group 1 (Gleason score 3 + 3), grade group ≥2 (Gleason score ≥3 + 4), grade group ≥3 (Gleason score ≥4 + 3)

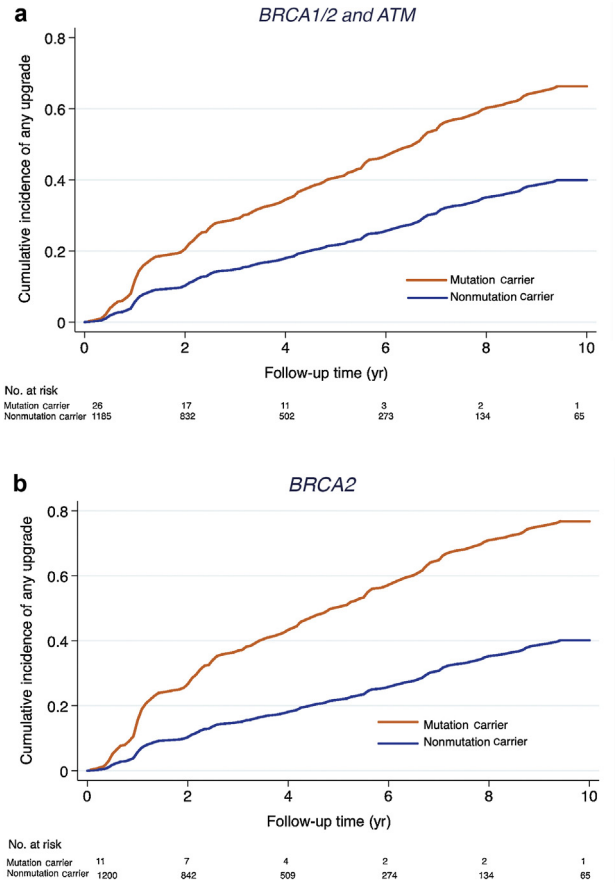

Fig. 1 – Cumulative incidence of upgrading on biopsies after the diagnostic biopsy in (A) carriers and noncarriers of mutations in *BRCA1/2* and/or *ATM*; (B) carriers and noncarriers of mutations in *BRCA2* only. Cumulative incidence based on competing risk analysis. Upgrading refers to any grade group (GG) or Gleason score higher than diagnostic biopsy GG irrespective of initial grade at biopsy.

SAS version 9.4 (Cary, NC, USA) and STATA version 13.1 (College Station, TX, USA).

3. Results

3.1. Cohort demographics

Cancer extent on prostate biopsy and the proportion of men with GG ≥2 at diagnosis was greater in the NS cohort than in the JH cohort (Table 1). Follow-up time and interval between diagnosis and upgrading was greater at JH than at NS. The JH cohort was more ethnically diverse than NS.

3.2. Carrier rates of pathogenic mutations

Considering all 1211 men, the carrier rates of mutations in the three-gene panel, and *BRCA2* alone, were significantly higher in those reclassified (3.8% and 2.1%, respectively) than those not reclassified (1.6% and 0.5%, respectively; $p = 0.04$ and 0.03, respectively; Table 2). Differences in mutation carrier rates in the three-gene panel for men that were reclassified or not were statistically significantly higher in the NS cohort than in the JH cohort ($p = 0.04$). The carrier rate for pathogenic mutations in the other 51 genes was not significantly different for men that were reclassified to a higher grade than those not reclassified after diagnosis (Supplementary Table 1).

3.3. Carrier rates by diagnostic GG and level of reclassification

The small number of men with a diagnostic GG 2 limited our ability to assess the relationship between mutation carrier status and diagnostic GG (Supplementary Table 2). The difference in carrier rates for *BRCA2* mutations between men who experienced reclassification or not was significantly greater for those that reclassified from GG 1 to ≥3 (4.1% vs 0.7%, $p = 0.01$) than those that reclassified from GG 1 to 2 (2.1% vs 0.56%; $p = 0.03$; Table 2).

3.4. Risk of GR by mutation carrier status for men with any diagnostic GG

Of 1211, 289 patients experienced any GR during a median follow-up of 3 (range, 1–6) yr on AS (Table 2). The cumulative incidence of any GR was significantly higher for carriers of mutations in the three-gene panel (Fig. 1A) and *BRCA2* alone (Fig. 1B) than for noncarriers (Table 3). The 2-, 5-, and 10-yr cumulative incidence of GR was 21%, 41%, and 67% versus 10%, 22%, and 40% in carriers versus noncarriers of mutations in the three-gene panel, respectively, and 27%, 50%, and 78% versus 10%, 22%, and 40% in carriers versus noncarriers of mutation in *BRCA2* alone, respectively (Fig. 1A and 1B). In a three-gene panel, 11 of 26 carriers of mutations versus 278 of 1185 noncarriers were reclassified by grade; adjusted HR = 1.96 (95% CI = 1.004–3.84, $p = 0.04$; Table 3). Reclassification occurred in six of 11 carriers of *BRCA2* mutations versus 283 of 1200 noncarriers; adjusted HR = 2.74 (95% CI = 1.26–5.96, $p = 0.01$).

3.5. Risk of GR by mutation carrier status for men diagnosed with GG 1

Of 1182 men with GG 1 at diagnostic biopsy, 283 (200 in JH and 83 in NS cohort) were reclassified to GG ≥2 and 98 (66 in JH and 32 in NS cohort) to GG ≥3 (Table 2). The cumulative incidence of any GR (GG ≥2) and reclassification to GG ≥3 was significantly higher for carriers of mutations in the three-gene panel (Fig. 2A and2B) than for noncarriers (Table 3). In the three-gene panel, 11 of 25 carriers of mutations versus 272 of 1157 noncarriers were reclassified from GG 1 at diagnosis to GG ≥2; adjusted HR = 1.98 (95% CI = 1.01–3.88, $p = 0.048$; Table 3), whereas five of 25 carriers and 93 of 1157 noncarriers were reclassified to GG ≥3; adjusted HR = 2.40 (95% CI = 1.01–5.71, $p = 0.046$). Reclassification to GG ≥2 occurred in six of 11 *BRCA2* mutation carriers compared with 277 of 1171 noncarriers (Table 3, Fig. 3A); adjusted HR = 2.44 (95% CI = 1.11–5.39, $p = 0.03$). Reclassification to GG ≥3 occurred in four of 11 *BRCA2* mutation carriers compared with 94 of 1171 noncarriers (Table 3, Fig. 3B); adjusted HR = 5.01 (95% CI = 2.22–11.61, $p = 0.001$).

Table 3 – Univariable and multivariable [a] relative risk estimates

Variable	No. of men	No. of events	Univariable analysis		Multivariable analysis	
			Relative risk (95% CI)	*p* value [b]	Relative risk (95% CI)	*p* value [b]
Any upgrading after diagnostic biopsy						
BRCA1/2 and *ATM*						
Mutation carrier	26	11	2.13 (1.10–4.13)	0.03	1.96 (1.004–3.84)	0.04
Nonmutation carrier	1185	278	1.00		1.00	–
BRCA2						
Mutation carrier	11	6	2.84 (1.11–7.26)	0.03	2.74 (1.26–5.96)	0.01
Nonmutation carrier	1200	283	1.00		1.00	–
Upgrading from GG1 to GG $\geq$ 3						
BRCA1/2 and *ATM*						
Mutation carrier	25	5	2.55 (1.05–6.18)	0.04	2.40 (1.01–5.71)	0.046
Nonmutation carrier	1157	93	1.00	–	1.00	–
BRCA2						
Mutation carrier	11	4	5.10 (1.86–13.93)	0.002	5.01 (2.22–11.61)	0.001
Nonmutation carrier	1171	94	1.00		1.00	–
Upgrading from GG 1 to GG $\geq$2						
BRCA1/2 and *ATM*						
Mutation carrier	25	11	2.17 (1.12–4.23)	0.02	1.98 (1.01–3.88)	0.048
Nonmutation carrier	1157	272	1.00		1.00	
BRCA2						
Mutation carrier	11	6	2.61 (1.03–6.61)	0.04	2.44 (1.11–5.39)	0.03
Nonmutation carrier	1171	277	1.00		1.00	

CI = confidence interval; HR = hazard ratio.
[a] Competing risk analysis adjusted for baseline risk factors of age, PSA density, number of cores with cancer, and Eigen values (genetic background).
[b] Comparison of risk of biopsy grade reclassification between mutation carriers and noncarriers using Fine and Gray model [19].

4. Discussion

AS is an option for men diagnosed with favorable-risk and intermediate-risk PCa [1,2]. However, there is a wide variation in selection of candidates for AS. In part, practice variation is due to uncertainty of the "true" disease phenotype because biopsy sampling can misclassify cancer grade and extent. Thus, some men will lose the opportunity for curative treatment either because of misclassification at diagnosis or disease progression during follow-up [3,4]. Consequently, there is growing interest in phenotypic and genotypic classification of PCas that inform management based on cancer biology.

Inherited germline DNA repair gene mutations are associated with an increased risk of PCa and a more aggressive biological behavior [8,9,21]. Thus, we hypothesized that men who qualified for AS would be at an increased risk of GR if they were carriers of germline mutations in repair genes. We found a significant association between mutation carrier status in a three-gene panel and the rate of GR among men who qualified for AS. Furthermore, the association between GR and mutation carrier status was strongest for *BRCA2* (six of 11 carriers vs 283 of 1200 noncarriers), and those men with *BRCA2* mutations that reclassified to GG >3 after diagnosis of GG 1 (four of 11 carriers vs 94 of 1171 noncarriers; Table 3). These men had a five-fold greater risk of GR than noncarriers. Given the association between cancer grade and cancer-specific outcomes in the absence of treatment, our findings suggest that AS could be associated with more risk for men with germ line mutations in *BRCA1/2* and *ATM*.

Our evaluation focused on *BRCA1/2* and *ATM* based on the findings that mutations in these genes are associated with an aggressive phenotype [8,10,12,13]. Na et al. [10] compared carrier rates of *BRCA1/2* and *ATM* germline mutations in 313 men who died of PCa and 486 with low-risk localized disease. They found significantly higher mutation carrier rates in the lethal phenotype (6.1%) than in those with localized disease (1.4%); additionally, mutation status was an independent predictor of lethal PCa and associated with earlier age at death and shorter survival.

The 2% overall carrier rate of *BRCA1/2* and *ATM* mutations in the current study (JH 1.8% and NS 3%; Table 2) is similar to the rate of *BRCA2* mutations in 2.3% of men with early onset disease before the age of 56 yr [22], and the carrier rate of mutations in *BRCA1/2* and *ATM* found in 1.4% of men with low-risk PCa undergoing treatment and enrolled in AS [10]. While the low carrier rate might limit the usefulness of this information in an AS cohort overall, it would be informative for an individual carrier of such a mutation.

The higher rate of germline mutations in the NS cohort than in the JH cohort in the current study is likely due to a greater proportion of men with higher-grade disease at diagnosis at NS but could also be affected by a larger Jewish population in Chicago than in Baltimore. Therefore, we adjusted for genetic background in our analysis.

Our findings and those of others suggest that men with germline mutations in *BRCA1/2* and *ATM* should factor the increased risk of harboring or developing a high-grade

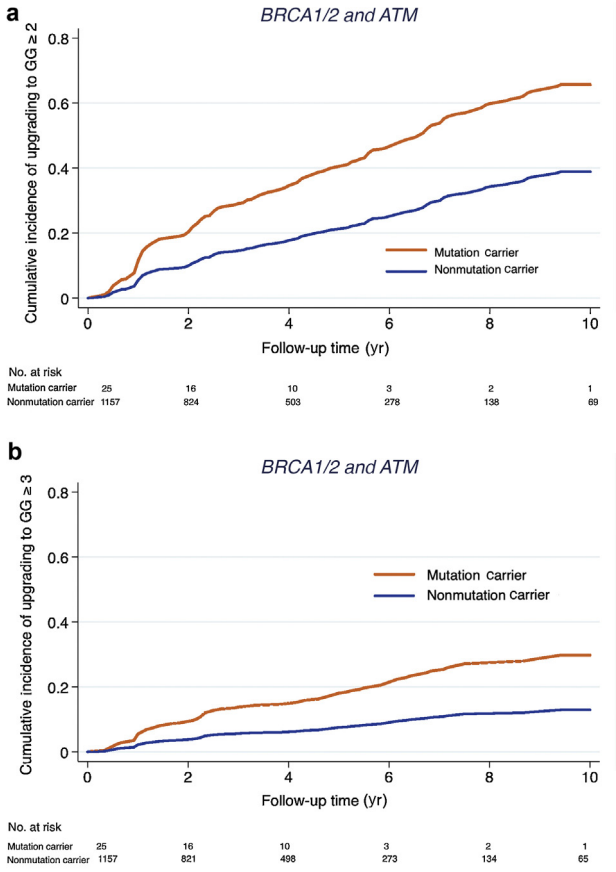

Fig. 2 – Cumulative incidence of upgrading after diagnostic biopsy among carriers and noncarriers of mutations in *BRCA1/2* and/or *ATM* who were initially diagnosed with grade group (GG) 1 (Gleason score 3 + 3) (A) upgrading after diagnostic biopsy to GG 2 or above (Gleason score 3 + 4 or above); (B) upgrading after diagnostic biopsy to GG 3 or above (Gleason score 4 + 3 or above). Cumulative incidence based on competing risk analysis.

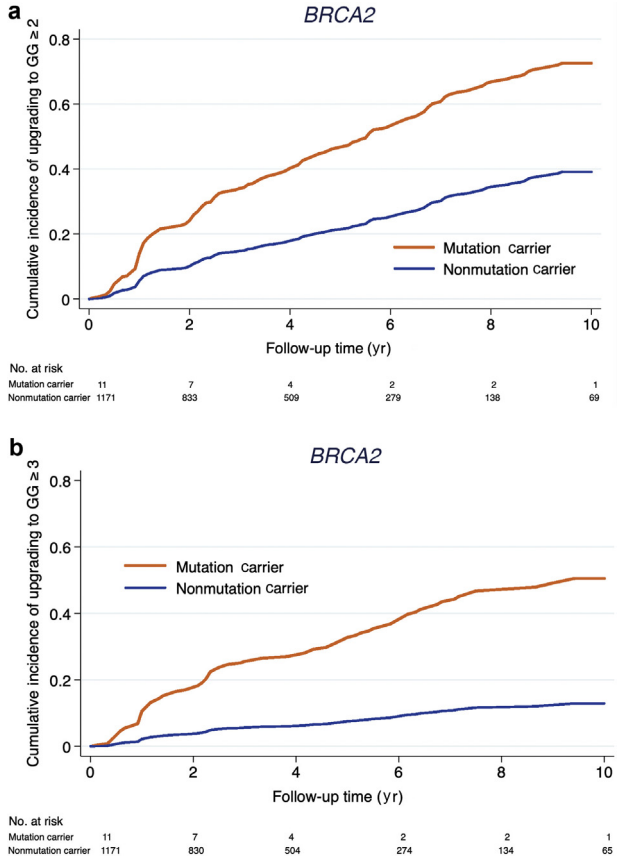

Fig. 3 – Cumulative incidence of upgrading after diagnostic biopsy among carriers and noncarriers of mutations in *BRCA2* only who were initially diagnosed with grade group (GG) 1 (Gleason score 3 + 3) (A) upgrading after diagnostic biopsy to GG 2 or above (Gleason score 3 + 4 or above); (B) upgrading after diagnostic biopsy to GG 3 or above (Gleason score 4 + 3 or above). Cumulative incidence based on competing risk analysis.

cancer in decision-making to enter AS. The current study cannot address the benefit of routine germline sequencing for men considering a surveillance strategy. However, based on the relationship between germline mutations in DNA repair genes and a more aggressive cancer phenotype [8,10,23], it would seem prudent for those with a family history of *BRCA1/2* mutations and a family history suggesting a higher likelihood of DNA repair gene mutations (eg, breast and ovarian cancer, Lynch syndrome, a family history of lethal PCa before age of 75 yr, death from PCa within 5–10 yr after diagnosis, and family history of pancreas cancer or male breast cancer). The decision to proceed with genetic testing may best be made in the context of a multidisciplinary setting that includes genetic counseling and a patient's personal preferences.

Our study has some limitations. First, there were small numbers of mutation carriers and short follow-up after diagnosis. Thus, the results are hypothesis-generating and require confirmation in larger cohorts. Second, our outcome of interest was GR which is an intermediate end point that may not reflect longer-term outcomes. Third, it is likely that given the time between diagnosis and GR in this cohort, that the initial biopsy misclassified a substantial proportion of men. Misclassification of risk could be reduced by image-guided biopsy and tissue expression assays, and these approaches could reduce the benefits of genetic testing as a marker of aggressive biology. Fourth, the study population was not ethnically diverse and results could differ in other populations. Finally, while mutations in other DNA repair genes were not associated with increased risk of GR, the frequency of these other mutation carriers was low, restricting definitive conclusions. This is particularly relevant for other gene mutations, such as 100delC CHEK2 [21], that may increase the risk of lethal disease.

5. Conclusions

Our study supports the findings of others that mutations in *BRCA1/2* and *ATM* are associated with a more aggressive PCa phenotype. Mutation status of *BRCA1/2* and *ATM* can help inform decisions between AS and curative intervention.

Author contributions: H. Ballentine Carter 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.

Study concept and design: Isaacs, Xu, Helfand, Carter.
Acquisition of data: Isaacs, Xu, Helfand, Carter, Novakovic.
Analysis and interpretation of data: All authors.
Drafting of the manuscript: All authors.
Critical revision of the manuscript for important intellectual content: All authors.
Statistical analysis: Mamawala.
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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.eururo.2018.09.021.

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