Published in final edited form as:

Eur Urol. 2017 May; 71(5): 740–747. doi:10.1016/j.eururo.2016.11.033.

Germline Mutations in *ATM* and *BRCA1/2* Distinguish Risk for Lethal and Indolent Prostate Cancer and are Associated with Early Age at Death

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Obtaining funding: Xu.

Administrative, technical, or material support: Zheng, Shah, Liu, Zhang. Supervision: Isaacs, Xu, Ding. Other: None

Financial disclosures: Jianfeng Xu certifies that all conflicts of interest, including specific financial interests and relationships and affiliations relevant to the subject matter or materials discussed in the manuscript (eg, employment/affiliation, grants or funding, consultancies, honoraria, stock ownership or options, expert testimony, royalties, or patents filed, received, or pending), are the following: None.

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Abstract

Background—Germline mutations in *BRCA1/2* and *ATM* have been associated with prostate cancer (PCa) risk.

Objective—To directly assess whether germline mutations in these three genes distinguish lethal from indolent PCa and whether they confer any effect on age at death.

Design, setting, and participants—A retrospective case-case study of 313 patients who died of PCa and 486 patients with low-risk localized PCa of European, African, and Chinese descent. Germline DNA of each of the 799 patients was sequenced for these three genes.

Outcome measurements and statistical analysis—Mutation carrier rates and their effect on lethal PCa were analyzed using the Fisher's exact test and Cox regression analysis, respectively.

Results and limitations—The combined BRCA1/2 and ATM mutation carrier rate was significantly higher in lethal PCa patients (6.07%) than localized PCa patients (1.44%), p = 0.0007. The rate also differed significantly among lethal PCa patients as a function of age at death (10.00%, 9.08%, 8.33%, 4.94%, and 2.97% in patients who died 60 yr, 61–65 yr, 66–70 yr, 71–75 yr, and over 75 yr, respectively, p = 0.046) and time to death after diagnosis (12.26%, 4.76%, and 0.98% in patients who died 5 yr, 6–10 yr, and > 10 yr after a PCa diagnosis, respectively, p = 0.0006). Survival analysis in the entire cohort revealed mutation carriers remained an independent predictor of lethal PCa after adjusting for race and age, prostate-specific antigen, and Gleason score at the time of diagnosis (hazard ratio = 2.13, 95% confidence interval: 1.24–3.66, p = 0.004). A limitation of this study is that other DNA repair genes were not analyzed.

Conclusions—Mutation status of *BRCA1/2* and *ATM* distinguishes risk for lethal and indolent PCa and is associated with earlier age at death and shorter survival time.

Patient summary—Prostate cancer patients with inherited mutations in *BRCA1/2* and *ATM* are more likely to die of prostate cancer and do so at an earlier age.

Keywords

Lethal prostate cancer; Germline; Mutation; DNA repair genes

1. Introduction

While over 1 111 700 men are diagnosed with prostate cancer (PCa) each year worldwide, a much smaller number (307 500) eventually die from this disease [1]. Understanding the inherited factors contributing to the progression of PCa to a lethal disease could have an important translational impact on the detection, diagnosis, and prognosis of this common

cancer. Specifically, a currently unmet clinical need is to be able to predict which men are more likely to develop a lethal PCa versus an indolent one.

The past 10 yr have seen substantial progress in elucidating molecular factors affecting PCa susceptibility with the identification of over 100 common genetic variants associated with an increased risk of PCa [2]. Although these factors provide robust markers of PCa risk overall, they are limited in distinguishing the risk for lethal versus indolent PCa [3,4].

One gene has emerged as a potentially specific driver of more aggressive PCa. In 1997, Sigurdsson et al [5] described the association of a deleterious founder mutation in *BRCA2* with aggressive PCa in Icelandic families. Subsequently, multiple studies confirmed the link between PCa and *BRCA2* emphasizing *BRCA2* as a strong risk factor [6–9]. Castro et al and others have described and characterized *BRCA2* as an important prognostic factor for aggressive PCa [10–19]; however, the mutation frequency was low and most estimates suggested that *BRCA2* accounted for a very small fraction of PCa (1–2%), even when early-onset family history-positive cases were examined [20–23]. In a seminal paper, Robinson et al [24] identified mutations in three DNA repair genes, *BRCA1/2*, and *ATM*, at a surprisingly high rate in men unselected for age at diagnosis or family history, but rather for aggressive disease.

More recently, Pritchard et al [25] demonstrated an elevated rate of mutations in a number of DNA repair genes in men with metastatic PCa. Importantly, the combined frequency of pathogenic mutations in a set of genes including *BRCA1/2* and *ATM* was higher than that reported in either the Exome Aggregation Consortium database of 53 000 unselected individuals or in the Cancer Genome Atlas database of men with clinically localized PCa. However, mixed racial populations and different sequencing technologies among study populations emphasize the need for confirmation of these findings.

In this study, we directly compared germline pathogenic mutations in *BRCA1/2* and *ATM* among lethal and indolent (low risk localized) PCa patients from three racial groups and assessed the effect mutational status on age at death in a large case-case PCa cohort.

2. Patients and methods

2.1. Study participants

This is a retrospective case-case study including 313 independent patients with lethal PCa and 486 independent patients with low risk localized PCa of European American, African American, and Chinese ancestry. Study participants were ascertained from patients undergoing PCa treatment in both the Brady Urological Institute and the Sidney Kimmel Comprehensive Cancer Center of the Johns Hopkins Medical Center, Baltimore, MD, USA (Hopkins), patients undergoing active surveillance at the John and Carol Walter Center for Urological Health, NorthShore University HealthSystem (NorthShore), as well as patients treated for PCa in the Department Huashan Hospital, Fudan University, Shanghai, China (Huashan). Lethal PCa in this study was defined as death due to metastatic PCa (obtained by death certificates and review of the patients' medical records). Localized PCa were patients diagnosed with low-risk disease, including PCa patients undergoing radical surgery with

pathological findings consistent with low-risk (pathological Gleason score 6, organ confined; Hopkins), PCa patients underwent active surveillance (NorthShore), and PCa patients met criteria of active surveillance (Huashan).

Clinical and demographic information of these patients, including age, race, prostate-specific antigen (PSA), Gleason score at time of PCa diagnosis, and years from diagnosis to death are summarized in Table 1. The Institutional Review Board at Johns Hopkins Medical Center, NorthShore University HealthSystem, and Huashan Hospital approved this study and when required, written informed consent was obtained from all study participants.

2.2. Sequencing of germline DNA

Whole-exome sequencing (WES) was performed on germline DNA derived from the blood of 129 lethal PCa patients at the PerkinElmer Next-generation Sequencing Service Laboratory. The Agilent SureSelect Human All Exon V5 was used to capture and enrich exome. Enriched libraries were sequenced using an Illumina HiSeq 2500 system. The mean sequencing depth of coverage was 71×. In addition, a customized next-generation sequencing panel targeting 222 cancer related genes was used to sequence the germline DNA of the remaining lethal PCa patients and all the indolent PCa patients. Probes for capturing exon regions (including 10 flanking intronic sequence) in these genes were manufactured by Roche NimbleGen. SeqCap EZ Library SR User's Guide (Roche, Pleasanton, CA, USA) was followed for library preparation and capture of targeted sequences. Paired-end sequencing of 2× 150 bp was performed on an Illumina MiSeq. Twelve individual libraries were multiplexed for a MiSeq flow cell. The mean sequencing depth of coverage was 135× overall and was 180×, 208×, and 219× for ATM, BRCA1, and BRCA2, respectively. All the targeted bases in these three genes were successfully sequenced (> 20×) in >99% samples. Sanger sequencing was used to confirm a subset of mutations identified in the WES and panel sequencing. Among the 19 samples analyzed using both WES and target panel, the concordance of called pathogenic and likely pathogenic mutations between the two sequencing methods was 100%.

2.3. Bioinformatics analysis

Paired-end reads were aligned to the GRCh37 version of the human genome using Burrows-Wheeler Aligner v0.7 to generate BAM files [26]. 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 version 3.2-2. Variant calling was performed with the Genome Analysis Toolkit 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 Exome Aggregation Consortium (http://exac.broadinstitute.org/), and clinical databases such as the Human Gene Mutation Database [27] and ClinVar [28]. Pathogenicity of variants is defined based on American College of Medical Genetics and Genomics criteria [29]. Specifically, pathogenic and likely pathogenic mutations are defined as: (1) all protein truncating mutations unless their allele frequency is 5% or higher in any racial group in population databases or is reported as benign or likely benign in the ClinVar, and (2) nonsynonymous

changes if their allele frequency is less than 5% and reported as pathogenic and likely pathogenic mutations in the ClinVar, (3) inframe-shift mutations affecting more than three amino acids are considered pathogenic mutations.

2.4. Statistical analysis

The frequency of pathogenic and likely pathogenic mutations was estimated for each gene in lethal cases and indolent cases and analyzed using the Fisher's exact test and logistic regression analysis. Time to death between mutation carriers and noncarriers was compared using Kaplan-Meier survival and Cox regression analysis adjusting for age, race, PSA level, and Gleason score at the time of diagnosis. Proportional hazard assumption was tested using log-log test and was met. Hazard ratio and its 95% confidence interval were calculated to estimate mortality risk. A type I error of 0.05 (two-sided) was used to define statistical significance.

3. Results

A total of 313 men who died from PCa and 486 low risk localized PCa patients were included in the study. Among them, 613, 119, and 67 were European American, African American, and Chinese men, respectively. Germline pathogenic and likely pathogenic mutations in ATM and BRCA1/2 detected in these study participants are described in Figure 1 and Supplementary Table 1. The frequency of mutations was 6.07% in lethal PCa patients, significantly higher than that observed in localized PCa patients (1.44%), p = 0.0007 (Table 2). Specifically, for BRCA2, mutations were found in 11 lethal PCa patients (3.51%) and four localized PCa patients (0.82%), p = 0.013. For ATM, mutations were found in six lethal PCa patients (1.92%) and two localized PCa patients (0.41%), p = 0.06. For BRCA1, mutations were found in two lethal PCa patients (0.64%) and one localized PCa patient (0.41%), p 0.99. The higher mutation frequency in lethal PCa compared with localized PCa patients was observed in all three racial groups. The difference was particularly striking in Chinese patients where three BRCA2 mutations and one ATM mutation were found in 22 lethal PCa patients (18.18%) while none were found in 45 localized PCa patients, p =0.0095. No patients in this study simultaneously carried a mutation in ATM and BRCA1 or -2.

Family history information was available in 669 patients (417 with negative family history and 252 with positive family history). The mutation carrier rates were similar in patients with a negative family history (12/417, 2.9%) and with a positive family history (8/252, 3.2%), p = 0.82.

In the entire cohort, mutation carrier status was not significantly associated with age at diagnosis; the median age at diagnosis for both mutation carriers and non-carriers was 64 yr, p = 0.53. However, mutation carrier status was significantly associated with more advanced PCa at time of diagnosis. Mutation carriers had a higher proportion of Gleason Score 7 (71%) than noncarriers (31%), p = 0.00009, and higher median PSA levels (7.90 ng/ml) than noncarriers (6.20 ng/ml), p = 0.048.

Mutation carrier status was significantly associated with progression of PCa. Among lethal PCa patients, the mutation carrier rates differed significantly as a function of age at death: 10.00%, 9.08%, 8.33%, 4.94%, and 2.97% in patients who died 60 yr, 61-65 yr, 66-70 yr, 71-75 yr, and >75 yr, respectively, p=0.046 (Table 3). No mutations were observed in 49 men dying from PCa over the age of 80 yr. The mutation carrier rates also differed significantly as a function of time to death after diagnosis (12.26%, 4.76%, and 0.98% in patients who died 5 yr, 6-10 yr, and >10 yr after a PCa diagnosis, respectively, p=0.0006; Table 3).

The survival analysis in the entire cohort revealed that men with pathogenic/likely pathogenic mutation of these three genes had a significantly shorter survival time (Fig. 2A). The median survival time after diagnosis was 5.0 yr in carriers and 16.0 yr in noncarriers (Log-rank $p = 3.7 \times 10^{-10}$). The association remained significant after adjusting for race and age, PSA, and Gleason Score (< 7 vs 7) at the time of diagnosis using the Cox regression analysis (hazard ratio = 2.13, 95% confidence interval: 1.24–3.66, p = 0.006).

Additional analyses were performed in subgroups of patients based on the disease status at time of diagnosis (Figs. 2B and 2C, Supplementary Table 2). Mutation carrier rate was highest in 122 lethal patients with the metastatic disease at time of diagnosis (8.2%), followed by 94 lethal PCa patients with localized disease at time of diagnosis (5.3%), and 486 PCa patients diagnosed with low-risk localized disease and remain localized at the time of the study (1.4%), $p = 5.4 \times 10^{-5}$. Mutation carrier status was a significant predictor of PCa-specific survival in patients diagnosed with the metastatic disease at time of diagnosis ($p = 3.8 \ 10^{-4}$), or in patients diagnosed with the localized disease at time of diagnosis (p = 0.0013).

Similar results were found when analyses were performed within each racial group (Tables 2 and 3), although most statistical significances were reached only in European Americans, the largest racial group examined in this study.

4. Discussion

In addition to confirming the major findings from previous studies on association of germline mutations of *BRCA2* and risk/progression of PCa, results from this study provide novel findings that further substantiate roles of germline mutations in these three DNA repair genes (*BRCA2*, *ATM*, and *BRCA1*) in distinguishing lethal from indolent PCa and predicting age of PCa-specific death. Our study is novel in several respects. Firstly, it is the first report analyzing germline mutations in these three genes in a large cohort of men who died of PCa, an important but understudied group of patients in previous studies [10,12,24,25]. Furthermore, few prior studies specifically included African American and Asians patients. While larger studies are obviously needed, the inclusion of men of African and Chinese descent in this study provides some initial indications of the importance of these genes in various populations.

Secondly, rather than relying on data from public databases, we performed sequencing, variant calling, and annotation in both groups of lethal and localized PCa patients thereby

facilitating an accurate comparison. This strategy reduced the likelihood that some of differences between the two groups of patients are results of technical aspects of sequencing methods (coverage and depth). Comparing mutation frequencies between lethal and localized PCa patients within each racial group reduced the likelihood that the observed difference is influenced by population stratification (ie, difference in mutation frequencies being confounded by different genetic backgrounds between the two comparison groups).

Thirdly, our results confirmed the association of mutations with short survival time [10,12–14,16,17,19], provided critical data for association of mutations with age of PCa-specific death, and importantly, for the first time, demonstrated a dose-response relationship between mutation carrier rate and age of death, as well as time to death. We found mutation carrier rate is highest among lethal PCa patients who died 60 yr (10.00%) or died within 5 yr after diagnosis of PCa (12.26%), and lowest among patients who died of PCa >75 yr (2.97%) or died >10 yr after diagnosis of PCa (0.98%). The mutation frequencies of the latter groups of lethal PCa patients were similar to those with localized PCa (1.23%). These results suggest that not all lethal PCa patients have a similar genetic basis and mutations in these three genes increase the risk for the most lethal form of PCa—dying younger and faster.

Our results, together with previous findings, may have important clinical implications. According to the National Comprehensive Cancer network guideline for PCa early detection, it is recommended to inquire about known *BRCA1/2* mutations in the family [30]. We propose to expand the inquiry to include whether there is a family member who died of PCa before age 75 yr. If they meet either condition, a genetic test of *BRCA1/2* and *ATM* is recommended. Another clinical implication of our findings is to consider inclusion of mutation carrier status as another important factor for decision making in active surveillance, although genetic studies specifically in active surveillance cohorts are needed for such indication. Mutation carriers may consider other types of interventional treatment.

Our data are comparable with that of recent findings of Pritchard et al [25] who observed a mutation frequency of 7.80% in these three genes in men with metastatic PCa. An inspection of the mutations found in the Pritchard et al study [25] reveals a significant fraction of founder mutations in both *BRCA1* and *BRCA2*, mainly from a single study site that is likely enriched for Ashkenazim, which may explain the slightly higher mutation frequency observed in their study [31,32]. In our study there were three founder mutation carriers, one in a lethal PCa patient and two in localized PCa patients.

We followed the general guideline of American College of Medical Genetics and Genomics to classify pathogenic and likely pathogenic mutations in all patients, regardless of disease status, as described in the Patients and methods section. Several of these mutations maybe debatable, including two *BRCA2* truncating mutations (NM_000059.3: c. 10094_10095insGAATTATATC; p.S3366Nfs*5 in Patient 9 and NM_000059.3: c. 10094_10095insGAATTATATC; p.V3365_S3366insNYI in Patient 17; Supplementary Table 1). Both mutations are in the last exon of the *BRCA2* transcript. The first mutation cosegregates with lethal PCa phenotype in this family (data not shown). If we remove these two mutation carriers, the main results of our study still hold; the frequency of pathogenic mutations was significantly higher in lethal cases (17/313, 5.43%) than localized cases

(7/486, 1.44%), p = 0.001, and mutation carriers remained an independent predictor of lethal PCa after adjusting for race and age, PSA, and Gleason Score at the time of diagnosis, p = 0.01. Another debatable mutation is ATMp.L950R (NM_000051.3: c.2849T>G). We called this mutation as likely pathogenic using our working criteria. In addition, this mutation is called likely pathogenic in ClinVar in Ataxia-telangiectasia families and Leu950 is located in an 11-amino acid sequence 942LHLHMYLML.LK952 that is conserved from human, cow, mouse, rat, to frog. Since this mutation was found in a localized prostate cancer patient, reclassifying this mutation to uncertain would not alter but rather slightly strengthen, our major results.

Limitations of our study include the small numbers of mutation carriers, even though a relatively large number of men who died from PCa were studied, reflecting the rarity of these mutations even in this enriched population. Indeed, despite finding an elevated rate of mutations in men with lethal PCa, the frequency of individual mutations is still quite low, necessitating large sample numbers to establish statistically significant, clinically meaningful associations and provide reliable estimates of mutation carrier rate. Genetic analyses limited to point mutations and small INDELs in this study, due to low confidence in detecting large-size of DNA deletions and gains using next-generation sequencing method, is another important limitation. We did not adjust our results for effects of differing treatments among the different sets of patients and how these treatments may affect outcome, although given the strong potential for a patient's mutational status to influence treatment, this question deserves much further study. Focusing on only the three most established DNA repair genes in this study is both limitation and strength. Many other DNA repair genes such as CHEK2 have been implicated in some studies [25]; however, evidence for their associations with risk and progression of PCa is still insufficient at this point for the primary purpose of this study which focuses on translation of more established germline findings. Additional research studies of other DNA repair genes and risk/progression of PCa in larger study populations are needed and undoubtedly underway. Finally, the full impact of these heterozygous germline mutations cannot be fully appreciated without knowing the somatic status of the other allele, as well as expression levels reflecting possible epigenetic silencing of nonmutated alleles.

5. Conclusions

In summary, our study provides additional evidence that the mutation status of *ATM* and *BRCA1/2* distinguishes the risk for lethal and indolent PCa and is associated with earlier age at death and shorter survival time. Together with previous studies, the consistent findings to date may have important clinical implications in genetic testing and decision making for PCa screening and treatment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The study is partially supported by the National Key Basic Research Program Grant 973 of China (2012CB518301), the Key Project of the National Natural Science Foundation of China (81130047), and the Rob Brooks Fund for Personalized Cancer Care at NorthShore University HealthSystem. We are also most grateful to the Ellrodt-Schweighauser family for establishing an Endowed Chair of Cancer Genomic Research at NorthShore University HealthSystem in support of Dr. Jianfeng Xu. We acknowledge Mr. Bob Aikens, Peter Bing, Warren Schwerin, and Frank Strang for their generous support of Dr. William Isaacs.

We also acknowledge Dr. Shan Zha for helpful discussions. We would thank John & Carol Walter family and Donald & Joan Rappeprt family for their support. Finally we thank all the patients for their participation in this study.

Funding/Support and role of the sponsor: The study is partially supported by the National Key Basic Research Program Grant 973 of China (2012CB518301), the Key Project of the National Natural Science Foundation of China (81130047), and the Rob Brooks Fund for Personalized Cancer Care at NorthShore University HealthSystem. Additional support was provided by the Commonwealth Foundation, SKCCC (to WBI).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.eururo.2016.11.033.

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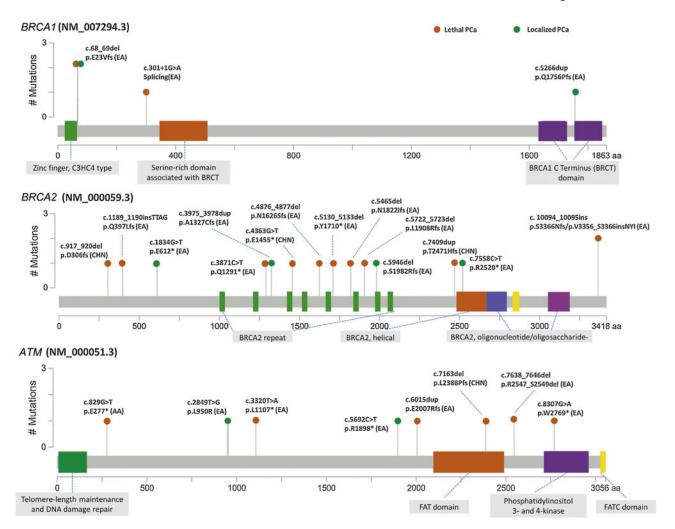
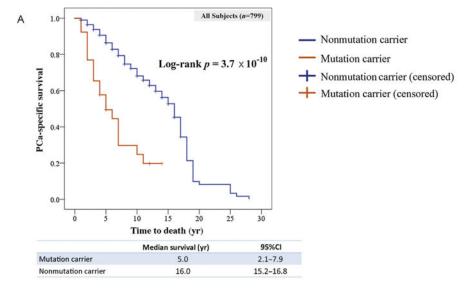
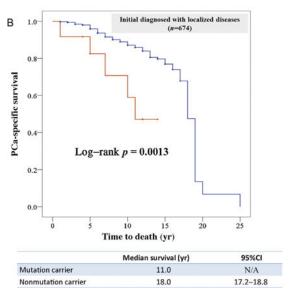
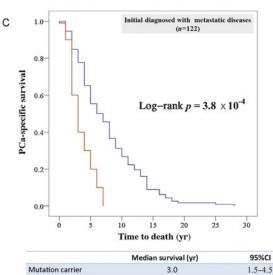


Fig. 1. Germline pathogenic and likely pathogenic mutations in *BRCA1/2* and ATM. Each mutation (complementary DNA position and resulting amino acid change) found in these three genes is presented by a lollipop plot. Mutations found in lethal and localized prostate cancer patients are coded in red and green, respectively. The y axis represents the total number of mutations identified.

AA = African Americans; BRCT = BRCA1 C-Terminal domain; CHN = Chinese; EA = European Americans; FAT = focal adhesion targeting; FATC = FRAP, ATM, TRRAP C-terminal; PCa = prostate cancer.







6.0

4.5-7.5

Fig. 2. Kaplan-Meier survival curves for mutation carriers and noncarriers (A) in the entire study cohort, (B) in patients diagnosed with localized prostate cancer at time of diagnosis, and (C) in the patients diagnosed with metastatic prostate cancer at time of diagnosis. PCa = prostate cancer.

Nonmutation carrier

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Table 1

Characteristics of study population

		All		European American	American	African A	African American	Chinese	iese
	Lethal $(n = 313)$	Localized $(n = 486)$	p value	Lethal $(n = 261)$	Localized $(n = 352)$	Lethal $(n = 30)$	Localized $(n = 89)$	Lethal $(n = 22)$	Localized $(n = 45)$
Age at diagnosis, yr (median, quartiles)	62.0 (57.0–68.0) 65.0 (8.7×10^{-5}	62.0 (57.0–68.0)	65.0 (64.0–67.0)	63.0 (54.8–68.0)	57.0 (53.0-60.0)	$(63.0-68.0) 8.7 \times 10^{-5} 62.0 \ (57.0-68.0) 65.0 \ (64.0-67.0) 63.0 \ (54.8-68.0) 57.0 \ (53.0-60.0) 68.5 \ (61.5-71.0) 71.0 \ (67.0-75.0) 63.0 \ (64.0-67.0) $	71.0 (67.0–75.0)
PSA, ng/ml (median, quartiles)	13.1 (6.4–62.1) 5.3 (3.	5.3 (3.8–7.7)	6.1×10^{-44}	6.1×10^{-44} 11.3 (6.0–43.8) 4.9 (3.7–7.0)	4.9 (3.7–7.0)	11.9 (7.2–85.0) 5.1 (3.5–6.6)	5.1 (3.5–6.6)	100 (22.5–306.6) 10.6 (8.2–14.1)	10.6 (8.2–14.1)
Proportion of Gleason Score 7 PCa (%)	222/276 (80.43) 22/485 (4.53)	22/485 (4.53)	2.8×10^{-14}	2.8×10^{-14} 128/228 (56.14) 22/351 (6.26)	22/351 (6.26)	21/27 (77.78) 0/89 (0)	(0) 68/0	19/21 (90.48)	0/45 (0)
Age at death, yr (median, quartiles)	72.0 (64.0–78.0)			72.0 (64.0–78.0) —	1	71.5 (63.2–76.5)	-	71.0 (64.0–73.3)	1

 $PCa = prostate \ cancer; \ PSA = prostate-specific antigen.$

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Table 2

Carrier rates of pathogenic/likely pathogenic mutations in BRCA1/2 and ATM

		All		Euro	European American	an	Afr	African American	an		Chinese	
	Lethal $(n = 313)$	Lethal Localized p value $(n=313)$ $(n=486)$ $(n=261)$ $(n=352)$ $(n=36)$ $(n=39)$ $(n=89)$ $(n=22)$ $(n=45)$	p value	Lethal $(n = 261)$	Localized $(n = 352)$	p value	Lethal $(n = 30)$	Localized $(n = 89)$	p value	Lethal $(n = 22)$	Localized $(n = 45)$	p value
BRCAI, n (%) 2 (0.64) 2 (0.41) 1.00	2 (0.64)	2 (0.41)	1.00		2 (0.77) 2 (0.57) 1.00 0 (0)	1.00	0 (0)	0 (0)		0 (0)	0 (0)	
BRCA2, n (%) 11 (3.51) 4 (0.82)	11 (3.51)	4 (0.82)	0.013	8 (3.07)	8 (3.07) 4 (1.12) 0.14	0.14	0 (0)	0 (0)		3 (13.64)	0 (0)	0.032
ATM, n (%)	6 (1.92)	6 (1.92) 2 (0.41) 0.06	90.0		4 (1.53) 2 (0.57) 0.41 1 (3.33) 0 (0) 0.25 1 (4.55)	0.41	1 (3.33)	0 (0)	0.25	1 (4.55)	0 (0)	0.34
AII, n (%)	19 (6.07)	$19 \; (6.07) \gamma \; (1.44)^a 0.0007 14 \; (5.36) \gamma \; (1.70)^a 0.041 1 \; (3.33) 0 \; (0) 0.25 4 \; (18.18) 0 \; (0)$	0.0007	14 (5.36)	$7 (1.70)^{a}$	0.041	1 (3.33)	0 (0)	0.25	4 (18.18)	0 (0)	0.0095

 $^{^{\}it a}$ One patient carries both BRCA1 and BRCA2 mutation.

Table 3

Carrier rates of pathogenic/likely pathogenic mutations in BRCA1/2 and ATM by age of onset and age of death

	N	(%) of pathogenic/likely	pathogenic mutation	s
	All	European American	African American	Chinese
All	19 (6.07)	14 (5.36)	1 (3.33)	4/22 (18.18)
Age of death (yr)				
60	4/40 (10.00)	4/33 (12.12)	0/5 (0)	0/2 (0)
60–65	5/55 (9.09)	4/48 (8.33)	0/3 (0)	1/4 (25.00)
65–70	3/36 (8.33)	2/29 (6.90)	0/3 (0)	1/4 (25.00)
70–75	4/81 (4.94)	2/58 (3.45)	0/11 (0)	2/12 (16.67)
>75	3/101 (2.97)	2/93 (2.15)	1/8 (12.50)	0/0 (-)
p value	0.046	0.014	_	_
Time to death, yr (from diagnosis)				
5	13/106 (12.26)	8/73 (10.96)	1/11 (9.09)	4/22 (18.18)
5–10	5/105 (4.76)	5/92 (5.43)	0/13 (0.00)	0/0 (0.00)
>10	1/102 (0.98)	1/96 (1.04)	0/6 (0.00)	0/0 (0.00)
Pvalue	0.0006	0.004	0.25	_