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Rare germline genetic variants and risk of aggressive prostate cancer

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Novelty and Impact:

Identifying which men at the time of prostate cancer diagnosis have, or will progress to, aggressive fatal disease remains an important clinical and public health issue. Germline gene

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panel testing offers opportunities for identifying men who carry *BRCA2* pathogenic variants who are at increased risk of aggressive disease. Our case-case study confirms this and provides further supportive evidence that men with pathogenic variants in *ATM* are also at increased risk of aggressive disease. The clinical relevance of the genetic variation identified in most of the other genes included in these gene panel tests remains uncertain and requires international attention and collaboration.

Abstract

Few genetic risk factors have been demonstrated to be specifically associated with aggressive prostate cancer (PrCa). Here, we report a case-case study of PrCa comparing the prevalence of germline pathogenic/likely pathogenic (P/LP) genetic variants in 787 men with aggressive disease and 769 with non-aggressive disease.

Overall, we observed P/LP variants in 11.4% of men with aggressive PrCa and 9.8% of men with non-aggressive PrCa (two-tailed Fisher's exact tests, P=0.28). The proportion of *BRCA2* and *ATM* P/LP variant carriers in men with aggressive PrCa exceeded that observed in men with non-aggressive PrCa; 18/787 carriers (2.3%) and 4/769 carriers (0.5%), P=0.004, and 14/787 carriers (0.02%) and 5/769 carriers (0.01%), P=0.06, respectively. Our findings contribute to the extensive international effort to interpret the genetic variation identified in genes included on gene-panel tests, for which there is currently an insufficient evidence-base for clinical translation in the context of PrCa risk.

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Introduction

A family history of prostate cancer (PrCa) is a well-established risk factor for developing the disease, indicating an important contribution of genetic risk factors in PrCa development. In a large twin study, Mucci *et al.* estimated that the heritability of prostate cancer was 57% [95% CI = 51-63%] and thus higher than that of breast (31% [95% CI = 11-51%]) and ovarian cancer (39% [95% CI = 23-55%]) [1]. To date, more than 150 common genetic variants have been reported and account for approximately 28% of the familial risk of PrCa [2-4]. A rare missense variant in *HOXB13* (c.251G>A; p.Gly84Glu) has been shown to be associated with increased risk of early-onset PrCa in the context of a family history of the disease [5-8]. This variant is estimated to account for ~5% of familial aggregation of PrCa [9]. Family observations and candidate gene approaches have shown that pathogenic variants in some DNA repair genes are associated with an increased risk of PrCa. Edwards *et al* reported that 2% of men diagnosed with PrCa under the age of 55 years carry pathogenic *BRCA2* variants that are associated with an 8.6-fold increased PrCa risk by age 65 years [10]. Men who carry a germline pathogenic variant in *BRCA1* are estimated to be at up to 4.5-fold increased risk of PrCa [11]. PrCa cases who carry pathogenic variants in *BRCA1* and *BRCA2* (combined) are more commonly aggressive, have increased nodal involvement and metastases, and have poorer survival [12-17]. Most recently, the IMPACT study has conducted single gene analyses that demonstrated significant association between mutation carrier status and younger age at onset and clinically significant disease for men with *BRCA2* pathogenic variants only [18]. PrCa arising in carriers of *MLH1*, *MSH2*, *MSH6* and *PMS2* germline pathogenic variants (Lynch syndrome) have been reported to be mismatch repair (MMR) deficient via immunohistochemistry [19]. Combined, men

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who carry a pathogenic variant in a MMR gene are reported to be at a 3.2-fold increased risk of PrCa, which has a notable contribution from variants in *MSH2* (5.8-fold increased risk) [19]. A recent study of *PMS2*-associated Lynch syndrome found no association with prostate cancer risk [20].

By comparing men with and without PrCa using a case-control design, most studies to date have searched for inherited genetic variants that predispose men to overall PrCa risk. However, the identification of germline genetic factors that can predict not only risk, but the clinical outcome for PrCa, if diagnosed, can both improve early diagnosis of potentially aggressive and lethal cases, and reduce overtreatment of indolent disease. A limited number of publications have focused on inherited genetic variants that distinguish between risk for aggressive (metastatic) and non-aggressive (low clinical grade) PrCa using a case-case design [4, 21].

Here, we report a case-case study of PrCa comparing the prevalence of germline pathogenic genetic variants in men with aggressive and non-aggressive disease. Since most of the genes currently implicated in PrCa susceptibility are also at least putatively involved in predisposition to breast and/or ovarian cancer and Lynch syndrome, we designed a panel targeting the coding regions of *ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, FANCM, HOXB13, MLH1, MRE11A, MSH2, MSH6, MUTYH, NBN, NF1, PALB2, PTEN, RAD50, RAD51C, RAD51D, RECQL, RNASEL, STK11* and *TP53,* and selected regions of *PMS2.* We applied massively parallel sequencing to screen the coding regions and proximal intron-exon junctions of these 26 genes, in men with aggressive PrCa and men with non-aggressive disease. We focused the analysis on rare pathogenic/likely pathogenic (P/LP) variants and assessed the number of carriers of such variants for each gene, and across all genes.

Materials and Methods

Subjects

Participants in this study were identified from i) the Melbourne Collaborative Cohort Study (MCCS), ii) the Aggressive Prostate Cancer (APC) study, iii) the Risk Factors for Prostate Cancer Study and iv) the Early-Onset Prostate Cancer Family Study [22-24].

Aggressive cases (n=787) were selected using the following criteria: PrCa as a cause of death (regardless of stage or Gleason score at diagnosis), or stage 4 (regardless of Gleason score) or stage 3 and Gleason score >= 8. Non-aggressive cases (n=770) were selected using the following criteria: stage 1 (T1/T2a) and Gleason score <=6 and age at diagnosis >=65 years; or stage 1 (T1/T2a) and Gleason score <=6 and age at diagnosis 55-64 years and >=10 years of follow-up; or stage 2 and Gleason score <=6, age at diagnosis >=65 years and >=10 years of follow-up. Germline DNA from these 1,557 participants was obtained from blood samples.

Gene-panel sequencing

The following 26 genes were included in the panel: *ATM (NM_000051.3), BARD1 (NM_000465.2), BRCA1 (NM_007294.3), BRCA2 (NM_000059.3), BRIP1 (NM_032043.2), CDH1 (NM_004360.3), CHEK2 (NM_007194.3), FANCM (NM_020937.2), HOXB13 (NM_006361.5), MLH1 (NM_000249.3), MRE11A (NM_005591.3), MSH2 (NM_000251.2), MSH6 (NM_000179.2), MUTYH (NM_001128425.1), NBN (NM_002485.4), NF1 (NM_000267.3), PALB2 (NM_024675.3), PMS2 (NM_000535.5), PTEN (NM_000314.4), RAD50 (NM_005732.3), RAD51C (NM_058216.2), RAD51D (NM_002878.3), RECQL (NM_002907.3), RNASEL (NM_021133.3), STK11 (NM_000455.4), TP53 (NM_000546.5).*

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Amplicon-based sequencing of the coding regions and proximal intron-exon junctions of 26 genes was performed using the *Hi-Plex2* protocol [25]. For *PMS2*, panel design avoided regions of homology with the pseudo-gene *PMS2CL*. A BED file of the targeted regions is available as Supplementary Data. Massively parallel sequencing (150 bp paired-end) was performed on the NextSeq550 platform (v2 chemistry, 2x150 bp) (Illumina, San Diego, CA, USA).

Paired-end reads were aligned to the reference genome (GRCh37) using bwa-mem 0.7.17 [26]. Target coverage was then calculated using bedtools [27]. Samples with ≥80% target bases covered at ≥ 50X sequencing depth were considered successfully sequenced. Applying these criteria, 787/787 aggressive and 769/770 non-aggressive PrCa cases progressed to variant calling. Variants were called using the Java version of VarDict in single sample amplicon-mode [28].

Variant annotation and classification

Variant calls were annotated using VEP and loaded into GEMINI, according to the authors recommendations [29, 30]. Variant nomenclature followed recommendations from the Human Genome Variation Society and used the reference transcripts listed above. Further analysis was restricted to variants that had a read depth ≥ 50X and a variant allele frequency ≥ 0.2, in the coding regions and within 20bp of the intron-exon boundaries of the genes included on the panel.

Rare variants were defined as variants reported by the Exome Aggregation Consortium (ExAC) with a minor allele frequency (MAF) ≤ 0.02 in the non-Finnish European population (NFE-non TCGA) [31].

Clinical interpretation was performed based on the American College of Medical Genetics and Genomics (ACMG) criteria and ClinVar (accessed 09/2019) [32, 33]. This study focused on rare, pathogenic or predicted loss of function (LoF) variants. All LoF variants (*ie.* nonsense, frameshifting indels and consensus splice site variants) were called pathogenic/likely pathogenic (P/LP) unless the LoF variant was classified as benign or likely benign in ClinVar (review status at least "2 stars"). Non-LoF variants were called P/LP when ClinVar assessment of pathogenicity and likely pathogenicity was "2 stars" or more, with multiple submitters and no conflicts.

Statistical analysis

The difference in the proportion of pathogenic mutation carriers for each gene, and across all genes, in the aggressive and non-aggressive PrCa cases was assessed using two-tailed Fisher's exact tests. No adjustment was made for multiple comparison.

Results

We assessed 26 genes involved in DNA repair and autosomal dominant cancer-predisposition syndromes in 787 aggressive PrCa and 770 non-aggressive cases. The clinical characteristics of the study participants are summarized in Table 1. Genetic testing was successfully performed for 787/787 (100%) participants with aggressive PrCa and 769/770 (99.9%) participants with non-aggressive PrCa. The median coverage per sample was 650X and 94.5% samples had ≥90% of the target bases covered at least at 50X.

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We identified a total of 95 P/LP variants in 90/787 (11.4%) men with aggressive PrCa and 82 P/LP variants in 75/769 (9.8%) men with non-aggressive PrCa (P=0.28, Table 2, Figure 1). All observed P/LP variants are reported in Supplementary Table 1.

Consistent with the literature, we found that *HOXB13*:c.251G>A; p.Gly84Glu was not associated with disease aggressiveness (10 non-aggressive PrCa cases, 8 aggressive PrCa cases, P=0.81). Our results support the established association of *BRCA2* P/LP variants with increased risk of aggressive disease. The proportion of *BRCA2* P/LP variant carriers in the aggressive PrCa group (2.3%, 18/787 men) exceeded that observed in the non-aggressive PrCa group (0.5%, 4/769 men) (P=0.004) (Table 2, Figure 1). There were 23 distinct *BRCA2* variants, 20 of which had expert panel-classification of Pathogenic in ClinVar. Two of the remaining *BRCA2* variants had a "2 stars"-classification as Pathogenic in ClinVar: *BRCA2*:c.631G>A and c.9117G>A. These variants have been shown to cause aberrant splicing that leads to skipping of exon 7 and exon 23 of the *BRCA2* mRNA, respectively [34-39]. *BRCA2*: c.8816delA has not yet received a ClinVar classification.

Other genes in which the proportion of P/LP variant carriers was higher in the aggressive PrCa group included *ATM* (14 (1.8%) men with aggressive PrCa versus five (0.7%) men with non-aggressive PrCa, respectively, P=0.06), *CHEK2* (10 (1.3%) men versus 5 (0.7%) men, respectively, P=0.30), and *BRCA1* (5 (0.7%) men versus 2 (0.3%) men, respectively, P=0.45) (Table 2, Figure 1).

Apart from *BRCA2,* the genes with the highest number of rare P/LP variants overall in both groups were *MUTYH* (n=29), *HOXB13* (n=19)*, RNASEL* (n=18) (Table 2, Figure 1). Genetic variants in *MUTYH* and *RNASEL* were less prevalent in men who had aggressive PrCa

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compared with men who had non-aggressive disease (P=0.27 and P=0.16, respectively) (Table 2, Figure 1). We observed one nonsense variant (*HOXB13*:c.327C>G) in a man with aggressive PrCa. Thirteen of the 18 carriers of P/LP variants in *RNASEL* carried *RNASEL*: c.793G>T, a nonsense variant, classified as pathogenic for PrCa susceptibility in ClinVar.

For five genes, P/LP genetic variants were observed only in men with non-aggressive PrCa but none of these results were statistically significant: *BRIP1* (one carrier, P=0.50)*, MRE11A, NF1* (two carriers each, P=0.25), *RAD50* and *RAD51C* (three carriers each, P=0.12) (Table 2).

We observed a total of 11 men with two or more distinct P/LP variants. In the aggressive PrCa group, four carried P/LP variants in two different genes (*BRCA2*:c.3778_3779delTT and *MUTYH*:c.536A>G; *CHEK2*:c.1100delC and *FANCM*:c.5101C>T; *ATM*:c.709dupA and *HOXB13*:c.327G>C; and *BRCA2*:c.6486_6489del and *RECQL*:c.1859C>G), and one man carried two pathogenic variants in *BRCA2* (*BRCA2*:c.631G>A and c.7008-2A>T). These two variants have been shown to be in *cis* [34, 35].

In the non-aggressive group, there were six men with two or more P/LP variants each; four carried P/LP variants in two distinct genes (*ATM*:c.8786+1G>A and *RAD51C*:c.1057_1066delCTGCATGTT; *MRE11A*:c.1927-2A>G and *RAD51C*:c.773G>A; *MUTYH*:c.536A>G and *RNASEL*:c.793G>T; *MUTYH*:c.536A>G and *RECQL*:c.1859C>G) and one man carried two *BRCA2* variants (*BRCA2*:c.3405C>A and c.8673_8674delAA). One man carried three P/LP variants (*CHEK2*:c.655delG, *NF1*:c.2033dupC and *NF1*:c.2186_2190_dupATAAC).

Discussion

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One of the major clinical challenges of PrCa is the clinical heterogeneity in the disease. Identifying genetic risk factors for disease aggressiveness may enable distinct clinical management strategies for men at high or low risk of developing this subtype, and thus, the development of a personalized approach to PrCa management. Men with such P/LP variants are not likely to be good candidates for management via active surveillance protocols, and conversely men without such variants may be able to be more confidently managed in such a fashion.

In a recent study involving 201 men with aggressive PrCa and 1,048 men with non-aggressive PrCa, Leongamornlert *et al.* presented evidence for P/LP variants in *BRCA2* to be associated with increased risk of aggressive PrCa [40]. The authors also found that carriers of P/LP variants in *MSH2* and *CHEK2* were at increased risk of developing aggressive disease. We observed too few P/LP *MSH2* variants to test the *MSH2* association with rigour (one carrier with aggressive PrCa and two carriers with non-aggressive disease). For *CHEK2*, their study found that only non-1100delC variants were associated with aggressive PrCa. We identified nine carriers of 1100delC in the aggressive group and three in the non-aggressive group. Although the findings were non-significant (1.1% and 0.4%, P=0.14), they are consistent with those from Wu *et al.* who reported a higher proportion of *CHEK2* c.1100delC carriers in men with lethal PrCa (1.28%) compared to those with low-risk disease (0.16%), P=0.003. [41]. Surprisingly, Leongamornlert *et al.* identified *ATM* P/LP variants only in the non-aggressive PrCa cases (seven carriers, 0.67%). Our study identified more men with a P/LP variant in *ATM* in the aggressive PrCa group than in the non-aggressive PrCa group (P=0.06). *ATM*:c.7271T>G, for which there is overwhelming evidence supporting an association with breast cancer risk similar

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in magnitude to *BRCA2* pathogenic variants, was identified twice in this study- both times in men with aggressive PrCa [42, 43].

In the field of hereditary breast and ovarian cancer, carriers of *BRCA1* and *BRCA2* pathogenic variants are known to be responsive to PARP inhibitors (PARPi), as well as platinum-based chemotherapy. The TOPARP study, a phase II trial evaluating men affected with PrCa treated with the PARPi olaparib, found that of the 16/49 study participants who responded to olaparib, six carried a germline pathogenic variant in a DNA repair gene (three in *BRCA2* and three in *ATM*) [44]. The other eight carried somatic variants in *BRCA1, CHEK2, PALB2, FANCA* and *HDAC2*. However, recently Marshall *et al*. have reported the outcome of a systematic review of 23 consecutive men with metastatic castration-resistant PrCa (mCRPC) carrying pathogenic germline and/or somatic variants in *BRCA1, BRCA2* or *ATM* treated with olaparib at three academic sites in the USA [45]. PSA responses to olaparib were achieved in 76% (13/17) of men with *BRCA1* or *BRCA2* compared to 0% (0/6) of men with *ATM* pathogenic variants who also had significantly shorter progression-free survival. Consistent with this observation, recent work applying CRISPR/Cas9 editing has provided evidence that *ATM* pathogenic variants can contribute to mCRPC progression via metabolic rather than DNA repair mechanisms [46].

The rarity of P/LP variants continues to challenge translational research as illustrated by Castro *et al.* who conducted a study in 419 men with mCRPC to evaluate the effect of pathogenic variants in DNA damage repair genes on response to taxane therapy [47]. Of these men, only 26 carried a germline pathogenic variant in *BRCA1, BRCA2* or *ATM*. Despite the study not reaching its primary end point, cause-specific survival was halved in *BRCA2* pathogenic

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variant carriers, suggesting that genetic testing of men with mCRPC may inform the selection of initial treatment.

Table 2 and Figure 1 illustrate the volume of genetic variation that is identified when using gene panel tests and that the data generated for the majority of genes included in these tests is currently uninterpretable. Interpretation of the genetic variation in genes such as *BRCA1, BRCA2, HOXB13* and *ATM*, for which data has been accumulating for some time, is more advanced but a considerable proportion of the variants remain of uncertain significance [18, 48, 49].

Although variants that can be classified as P/LP are rare, when considered in the context of commercial multigene testing for prostate cancer susceptibility, it is clear that the number of men to whom this information could be clinically relevant is not inconsequential. For example, Giri *et al.* report that 11% of men undergoing multigene testing for prostate cancer susceptibility (unselected for metastatic disease) had pathogenic variants in DNA repair genes that have implications for therapeutic management and cascade testing [50].

Recognised gaps in knowledge currently include cost-effectiveness of genetic testing for PrCa susceptibility and whether the strategies for testing should include breast cancer family history information and/or Gleason score [50].

Further extensive internationally coordinated studies are required to confidently classify rare pathogenic variants identified in multigene-testing. Evidence is accumulating in relation to the use of genetic information about *ATM* in the clinical management of men with prostate cancer. Much more data is required to interpret variation in *CHEK2* (observed in 1-2.2% of affected

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men (this report and [50]) and the many other genes currently included on commercial multi-gene testing panels for which there is currently an insufficient evidence-base for clinical translation.

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Table 1: Clinical characteristics of the participants to this study.

Study	Aggressive PrCa cases		Non-aggressive PrCa cases	
	N	%	N	%
APC[a]	257	32.7%	24	3.1%
EOPCFS[a]	185	23.5%	99	12.9%
MCCS[a]	166	21.1%	442	57.5%
RFPCS[a]	179	22.7%	204	26.5%
Age at diagnosis (years)				
<60	250	31.8%	97	12.6%
60-64	137	17.4%	92	12.0%
65-69	235	29.9%	298	38.8%
≥70	165	21.0%	282	36.7%
Size of the primary tumour (T stage)				
T1	290	36.8%	666	86.6%
T2	18	2.3%	103	13.4%
T3	341	43.3%	0	0.0%
T4	31	3.9%	0	0.0%
Unknown	107	13.6%	0	0.0%
Spread to lymph nodes (N stage)				
No	743	94.4%	769	100.0%
Yes	44	5.6%	0	0.0%
Presence of metastasis (M Stage)				
No	751	95.4%	769	100.0%
Yes	36	4.6%	0	0.0%
Gleason score				
2	2	0.3%	20	2.6%
3	1	0.1%	24	3.1%
4	5	0.6%	98	12.7%
5	16	2.0%	126	16.4%
6	47	6.0%	501	65.1%
7	132	16.8%	0	0.0%
8	198	25.2%	0	0.0%

9	238	30.2%	0	0.0%
10	25	3.2%	0	0.0%
Unknown	123	15.6%	0	0.0%
Died with prostate cancer as the cause of death				
Yes	468	59.5%	0	0.0%
No	319	40.5%	769	100.0%
Total	**787**		**769**	

[a] APC, Aggressive Prostate Cancer study; EOPCFS, Early-Onset Prostate Cancer Family Study; MCCS, the Melbourne Collaborative Cohort Study; RFPCFS, Risk Factors for Prostate Cancer Study

[b] All men with unknown T stage died of prostate cancer

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Table 2: Number of carriers of rare pathogenic/likely pathogenic variants observed per gene, in the aggressive (n=787 men) and non-aggressive (n=769 men) PrCa groups. In the aggressive PrCa group, five men each carried two distinct P/LP variants. In the non-aggressive PrCa group, there were five men who carried two P/LP variants each, and one man who carried three P/LP variants.

Gene	Aggressive PrCa cases[a]	Non-aggressive PrCa cases[a]	p-value[b]
ATM	14	5	0.06
BARD1	1	1	1.00
BRCA1	5	2	0.45
BRCA2	18[c]	4[c]	<0.01
BRIP1	0	1	0.49
CDH1	0	0	-
CHEK2	10	5	0.30
FANCM	4	3	1.00
HOXB13	11	8	0.65
MLH1	0	0	-
MRE11A	0	2	0.24
MSH2	1	2	0.62
MSH6	3	1	0.62
MUTYH	12	17	0.35

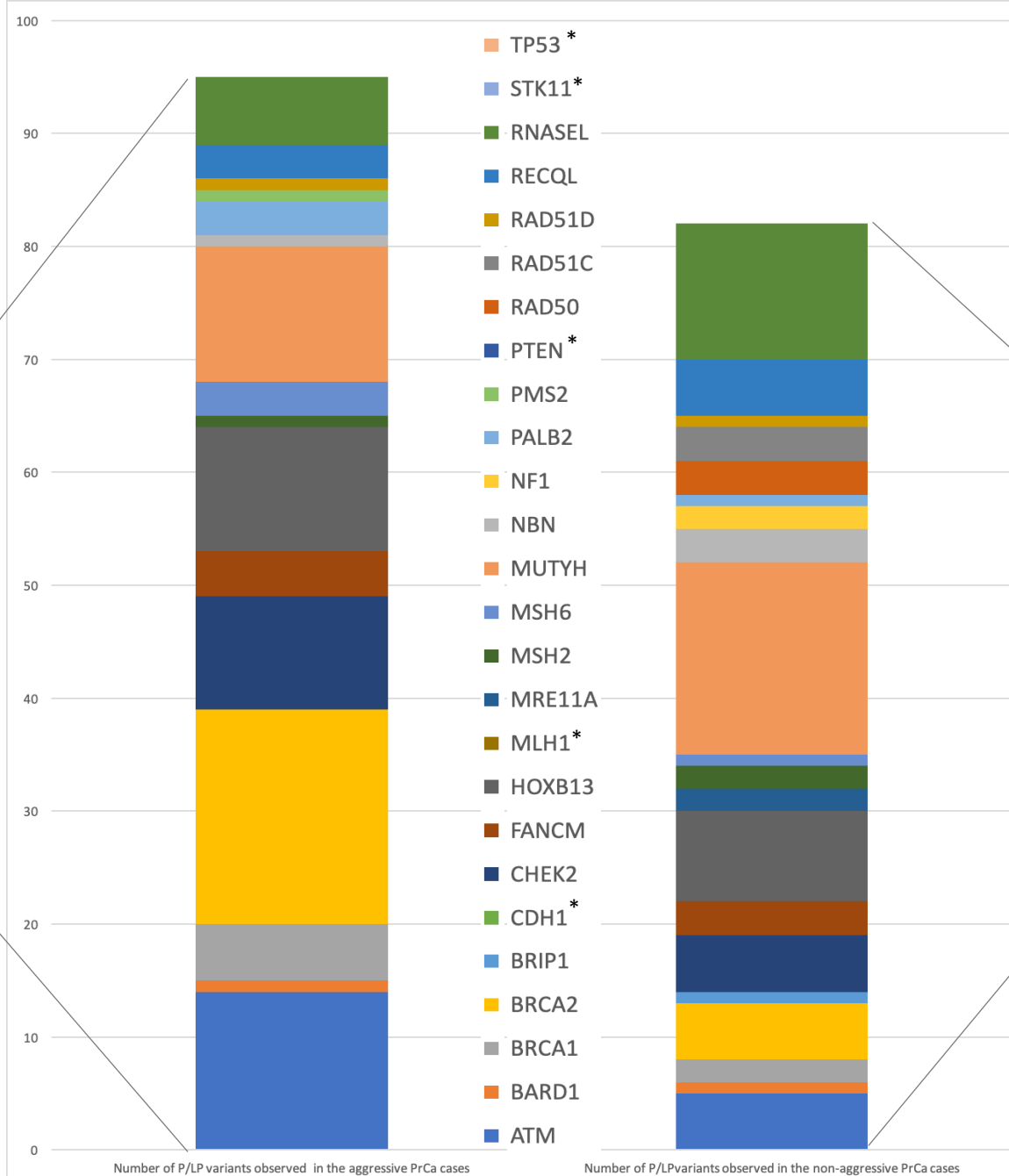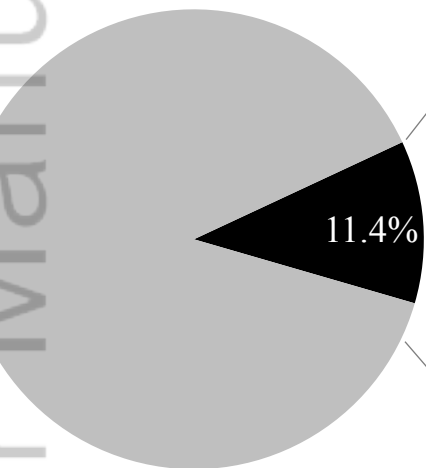|			
NBN	1	3	0.37
NF1	0	1[c]	0.49
PALB2	3	1	0.62
PMS2	1	0	1.00
PTEN	0	0	-
RAD50	0	3	0.12
RAD51C	0	3	0.12
RAD51D	1	1	1.00
RECQL	3	5	0.50
RNASEL	6	12	0.16
STK11	0	0	-
TP53	0	0	-

[a] Carriers of P/LP: pathogenic or likely pathogenic variants: rare loss-of-function variants (excluding those predicted to be benign in ClinVar), and rare non-loss-of-function variants that have a "2-star" classification as "pathogenic" in ClinVar (accessed September 2019). One man in each group carried two *BRCA2* P/LP variants. One man in the non-aggressive PrCa group carried two P/LP in *NF1*.

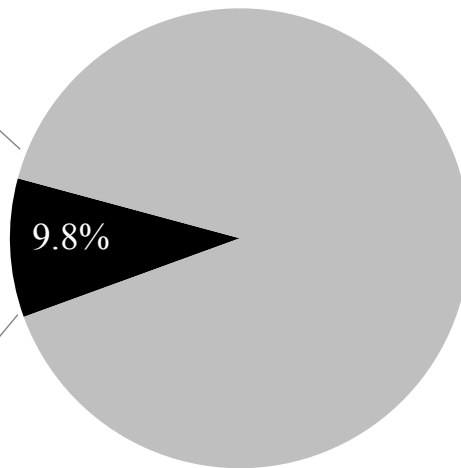[b] Two-tailed Fisher's exact test

[c] One man was found to carry two P/LP variants in this gene.

Figure 1: Pie charts representing the proportion of carriers of a pathogenic/likely pathogenic (P/LP) genetic variant identified in the 26 gene-panel in A) the aggressive PrCa (n=787 men) and B) the non-aggressive PrCa (n=769 men) groups. The bar plots represent the number of P/LP variants observed per gene in each group. * denotes genes in which no P/LP variant was identified.

A)

B)

11.4%

9.8%

TP53 *
STK11*
RNASEL
RECQL
RAD51D
RAD51C
RAD50
PTEN *
PMS2
PALB2
NF1
NBN
MUTYH
MSH6
MSH2
MRE11A
MLH1*
HOXB13
FANCM
CHEK2
CDH1 *
BRIP1
BRCA2
BRCA1
BARD1
ATM

Number of P/LP variants observed in the aggressive PrCa cases

Number of P/LPvariants observed in the non-aggressive PrCa cases

Most studies on prostate cancer (PrCa) have searched for inherited genetic variants that predispose men to overall PrCa risk. This study compares the prevalence of germline pathogenic genetic variants in men with aggressive and non-aggressive PrCa. The results confirm that germline gene panel testing allows identifying men who carry *BRCA2* pathogenic variants with increased risk of aggressive disease. Men with pathogenic variants in *ATM* were also at increased risk of aggressive disease. The findings contribute to the interpretation of the genetic variation identified in gene-panel tests and the evidence base for its clinical translation in the context of PrCa risk.