Germline sequencing DNA repair genes in 5,545 men with aggressive and non-aggressive prostate cancer

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ABSTRACT

BACKGROUND: There is an urgent need to identify factors specifically associated with aggressive prostate cancer (PCa) risk. We investigated whether rare pathogenic, likely pathogenic, or deleterious (P/LP/D) germline variants in DNA repair genes are associated with aggressive PCa risk in a case-case study of aggressive versus non-aggressive disease. **METHODS:** Participants were 5,545 European-ancestry men, including 2,775 non-aggressive and 2,770 aggressive PCa cases, which included 467 metastatic cases (16.9%). Samples were assembled from 12 international studies and germline sequenced together. Rare (minor allele frequency<0.01) P/LP/D variants were analyzed for 155 DNA repair genes. We compared single variant, gene-based, and DNA repair pathway-based burdens by disease aggressiveness. All statistical tests are two-sided. **RESULTS**: *BRCA2* and *PALB2* had the most statistically significant gene-based associations, with 2.5% of aggressive and 0.8% of non-aggressive cases carrying P/LP/D *BRCA2* alleles (OR=3.19, 95% CI=1.94 to 5.25, P=8.58x10^{-7}) and 0.65% of aggressive and 0.11% of non-aggressive cases carrying P/LP/D *PALB2* alleles (OR=6.31, 95% CI=1.83 to 21.68, P=4.79x10^{-4}). *ATM* had a nominal association, with 1.6% of aggressive and 0.8% of non-aggressive cases carrying P/LP/D *ATM* alleles (OR=1.88, 95% CI=1.10 to 3.22, P=.02). In aggregate, P/LP/D alleles within 24 literature-curated candidate PCa DNA repair genes were more common in aggressive than non-aggressive cases (carrier frequencies=14.2% versus 10.6%, respectively; P=5.56x10^{-5}). However, this difference was statistically non-significant (P=.18) upon excluding *BRCA2*, *PALB2*, and *ATM*. Among these 24 genes, P/LP/D carriers had a

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1.06-year younger diagnosis age (95% CI=-1,65 to 0.48, P=3.71x10^{-4}).

CONCLUSIONS: Risk conveyed by DNA repair genes is largely driven by rare P/LP/D alleles within *BRCA2*, *PALB2*, and *ATM*. These findings support the importance of these genes in both screening and disease management considerations.

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Prostate cancer (PCa) is the second leading cause of cancer death in the United States and fifth worldwide among men[1]. The five-year cancer-specific survival rate of men diagnosed with localized or regional PCa is nearly 100%, with those diagnosed with higher Gleason grade disease requiring more aggressive treatment. However, only ~30% of men diagnosed with metastatic PCa survive beyond five years[2]. In order to reduce both the number of deaths due to PCa and overtreatment of lower risk patients, it is critical to identify men at high risk of aggressive disease.

Multiple lines of evidence support a genetic contribution to aggressive PCa risk, including concordance of PCa survival duration between fathers and sons[3], familial aggregation of incident and fatal PCa[4, 5], and several genomic regions implicated by linkage studies of aggressive PCa[6-10]. However, the specific variants and genes implicated by linkage studies have yet to be identified, and few common variants have been associated with risk of aggressive as opposed to non-aggressive PCa[11, 12]. An important component of the genetic architecture of aggressive PCa may include multiple rare variants, which represent a sizable spectrum of human genetic variation yet to be comprehensively examined for aggressive disease.

Germline sequencing studies have reported that rare pathogenic and deleterious variants within DNA repair genes may predispose individuals to earlier PCa onset[13, 14], aggressive PCa[15-20], and response to PCa treatment[21, 22]. Among these studies, *BRCA2* is the most consistently reported gene, with evidence also reported for *ATM*, *CHEK2*, *MSH2*, and *NBN*, which are typically associated with increased aggressive PCa risk[13-22]. Due to the extreme rarity of pathogenic variants, larger sample sizes are needed to identify genes with statistically significant and consistent

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associations. Guidelines now recommend germline genetic testing for a panel of DNA repair genes at the time of initial PCa diagnosis for men with a family history or high-risk, regional, or metastatic PCa to inform disease management[23]; identifying the specific genes that impact aggressive disease risk would likely improve the clinical utility of such testing, which in the future could be offered prior to the diagnosis of PCa to inform screening decisions. However, previous studies have focused on a small number of candidate DNA repair genes and whole-exome sequencing studies have been conducted in small samples[15, 24]. A large-scale investigation of DNA repair genes in aggressive PCa has yet to be conducted.

Here we examined the involvement of rare pathogenic, likely pathogenic, and deleterious (P/LP/D) germline variants within a comprehensive panel of 155 DNA repair genes in PCa using a case-case investigation of 5,545 men of European ancestry comparing aggressive PCa (death due to PCa, metastatic disease, stage T4, or stage T3 and Gleason ≥8 tumors) with non-aggressive PCa cases (stages T1/T2 and Gleason ≤6 tumors). In addition to single variant associations, we tested gene- and pathway-based associations to examine the aggregate effect of rare P/LP/D variants on aggressive PCa and age at disease diagnosis.

METHODS

Participants and Genetic Sequencing

After excluding 18 men whose DNA samples failed quality control, 5,545 men of European ancestry selected from 12 large epidemiological studies across Australia, Finland, the United Kingdom, the United States, and Sweden were included in analyses.

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Participants were selected without knowledge or suspicion of genetic alleles carried (see **Supplementary Methods** for study recruitment details and sample quality control). Of these, 2,775 had non-aggressive PCa and 2,770 had aggressive PCa. Aggressive cases were men who either died from PCa or had metastatic disease, stage T4, or both stage T3 and a Gleason score ≥8 at diagnosis. Non-aggressive cases were men diagnosed with localized disease (stage T1/T2) and Gleason ≤6 tumors (71.3% of non-aggressive cases additionally had follow-up indicating that they were alive and without recurrence for ≥10 years). Variants within DNA repair genes were extracted from whole-exome sequencing data generated at the Center for Inherited Diseases Research with 56X mean targeted exon coverage (details in **Supplementary Methods**). All participants provided informed consent, and study protocols were approved by respective Institutional Review Boards.

DNA Repair Gene and Pathway Selection

DNA repair pathways were based on previous curations[25-28] and included homologous recombination/Fanconi anemia (HR/FA), ATM signaling (ATM), base excision repair (BER), nucleotide excision repair (NER), non-homologous end joining (NHEJ), mismatch repair (MMR), RECQ helicase family (RECQ), translesion synthesis (TLS), cross-link repair (XLR), and other miscellaneous DNA repair genes with functions including endonuclease/exonuclease activity and modification of chromatin structure (Other). From these curations and another DNA repair gene investigation[16], we identified 194 genes, of which 188 were sequenced and 155 contained variants meeting the inclusion criteria of our study (**Supplementary Table 1**). We also curated a

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candidate subset of 24 DNA repair genes based on previous literature supporting an association between germline variants in these genes and PCa risk or disease aggressiveness[13-16, 18, 21, 29] (**Supplementary Table 1**).

Pathogenic, Likely Pathogenic, and Deleterious Variant Definition

P/LP/D variants analyzed were rare (minor allele frequency<0.01) and had either a) a Variant Effect Predictor (VEP) Impact score of “high”[30], representing variants with deleterious (protein truncating or splice altering) functional consequences, or b) a Pathogenic or Likely Pathogenic ClinVar classification[31] to identify known pathogenic variants, including non-synonymous substitutions. We excluded variant c.9976A>T (rs11571833) in *BRCA2*, as it is a known low/moderate PCa risk variant[32].

Statistical Analyses

Single variant, gene-based, and pathway-based analyses were performed for aggressive versus non-aggressive PCa, metastatic versus non-aggressive PCa, and age at PCa diagnosis. As a secondary analysis, we assessed lethal (i.e., death from PCa) versus non-aggressive PCa. Single variants were analyzed using Firth logistic regression models[33] and the likelihood-ratio test (LRT). Gene-based and pathway-based analyses were performed by comparing P/LP/D carriers to non-carriers. Carrier status was compared between aggressive statuses using logistic regression models and tested for associations with age at diagnosis using linear regression models, with P-values calculated using LRT. Gene-based analyses excluded genes with five or fewer carriers of qualifying variants.

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Analyses included covariates for study, country, age at PCa diagnosis, and three principal components of ancestry to account for potential population stratification. Analyses of individual variants, genes, and pathways were corrected for multiple testing for each outcome using the Benjamini-Hochberg[34] adjustment. An adjusted P<.05 was considered statistically significant, while an unadjusted P<.05 was considered nominally statistically significant (P-values described within the Results section are unadjusted). All tests of statistical significance are two-sided. Top findings for each outcome were further investigated in analyses stratified by age at PCa diagnosis (<60 and ≥60 years of age), PCa family history (available for 79.2% [n=4,390] of participants), and country. Top findings were also further investigated comparing non-aggressive cases with subgroups of non-metastatic aggressive cases, including those diagnosed with 1) T1/T2 and Gleason <8, 2) T1/T2 and Gleason ≥8, 3) T3/T4 and Gleason <8, and 4) T3/T4 and Gleason ≥8 tumors. Analyses investigating age at diagnosis excluded 543 non-aggressive Australian participants, as the selection criterion applied to these samples included age at diagnosis (**Supplementary Methods**).

RESULTS

Participants

Of aggressive PCa cases, 74.1% (n=2,052) died due to PCa, 16.9% (n=467) had metastatic disease, 67.2% (n=1,862) had a Gleason score ≥8, and 69.7% (n=1,931) had stage T3 or T4 (**Table 1**). Of cases that died due to PCa, only 11.5% (n=319) had stage T1/T2 disease and Gleason <8 tumors at diagnosis. Aggressive cases were

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younger at diagnosis than non-aggressive cases (66.1 years [SD=8.8] versus 67.5 [SD=7.0], respectively).

Aggressive versus Non-Aggressive PCa

Among the 155 DNA repair genes, 858 P/LP/D variants were identified in the sample of 5,545 men (**Supplementary Figure 1**, **Supplementary Table 2**), which included 289 P/LP/D variants in the 24 candidate genes (**Figure 1**, **Supplementary Figure 2**). Owing to their rare frequencies, associations between single P/LP/D variants and aggressive PCa were statistically non-significant (**Supplementary Figure 3**, **Supplementary Table 3**).

BRCA2 and *PALB2* had the strongest gene-based associations with aggressive PCa (**Table 2**, **Supplementary Table 4**, and **Supplementary Figure 4**). We observed that 2.5% of aggressive and 0.8% of non-aggressive cases carried P/LP/D *BRCA2* alleles (OR=3.19, 95% CI=1.94 to 5.25, P=8.58×10^{-7}), while 0.65% of aggressive and 0.11% of non-aggressive cases carried P/LP/D *PALB2* alleles (OR=6.31, 95% CI=1.83 to 21.68, P=4.79×10^{-4}). *ATM* was nominally associated with aggressive PCa, with 1.6% of aggressive and 0.8% of non-aggressive cases carrying P/LP/D *ATM* alleles (OR=1.88, 95% CI=1.10 to 3.22, P=.02). Effects of these three genes were similar or only slightly larger when comparing metastatic cases with non-aggressive cases (**Table 2**). While six genes were nominally associated with metastatic disease, none were statistically significant after adjusting for multiple testing (**Supplementary Table 4**, **Supplementary Figure 4**). Associations with lethal PCa were similar in magnitude to aggressive disease, with slightly stronger effects (**Supplementary Table 4**,

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Supplementary Figure 4). Carrier frequencies and effects of the candidate PCa genes by disease aggressiveness are shown in **Figure 2A-B**.

In aggregate, P/LP/D alleles within the 155 DNA repair genes were more common in aggressive than non-aggressive PCa cases (carrier frequency=36.4% versus 33.1%, respectively; P=.03) but did not statistically significantly differ between metastatic and non-aggressive cases (P=.17; **Figure 2C-D, Supplementary Table 5**). Larger differences were observed in the 24 candidate PCa genes, with non-aggressive cases having a statistically significantly lower carrier frequency (10.6%) than aggressive cases (14.2%; $P=5.56 \times 10^{-5}$) and metastatic cases (15.4%; $P=3.61 \times 10^{-4}$). Upon removing the 24 candidate genes from the 155 DNA repair genes, the remaining 131 genes were not associated with aggressive PCa risk (**Figure 2C-D**). Further, the observed association with the 24 candidate genes was determined only by a small number of genes—upon sequentially removing genes with the strongest risk-increasing effects, the remaining genes had no aggregate effect on aggressive disease (excluding *BRCA2*, *PALB2*, and *ATM*, P=.18; excluding *BRCA2*, *PALB2*, *ATM*, *MLH1*, *CHEK2*, *MUTYH*, and *MSH2*, P=.59). Removing these genes similarly led to decreased aggregate effects on metastatic disease, with a residual statistically non-significant effect observed after excluding the seven genes (OR=1.10, 95% CI=0.69-1.74, P=.69). P/LP/D alleles in *BRCA2*, *PALB2*, and *ATM* were found in 1.7% of non-aggressive versus 4.7% of aggressive ($P=5.46 \times 10^{-10}$) and 5.1% of metastatic cases ($P=6.54 \times 10^{-5}$; **Supplementary Table 5**).

The HR/FA pathway was the only pathway with a statistically significant association, with carriers of P/LP/D HR/FA alleles having 1.27-fold increased risk of

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PCa death (95% CI=1.05 to 1.53, P=.004); however, this association was statistically non-significant after excluding *BRCA2* (P=.47; **Supplementary Table 6**, **Supplementary Figure 5**). The NER and MMR pathways were associated with a 1.48-fold and 1.29-fold increased risk of aggressive PCa, respectively, although neither was statistically significant (95% CI=1.00 to 2.18, P=.045 and 95% CI=0.95 to 1.76, P=.10, respectively).

Age at PCa Diagnosis

P/LP/D alleles within *BRCA2*, *NBN*, *ATM*, and *CCNH* had nominal (P<.05) associations with younger age at diagnosis; however, none were statistically significant after correcting for multiple testing (**Supplementary Figure 6A-B**, **Supplementary Table 7**). Carrying P/LP/D alleles within the 155 DNA repair genes was associated with a 0.59-year younger age at PCa diagnosis (95% CI=-1.00 to -0.19, P=.004; **Supplementary Table 5**). Upon removing the 24 candidate PCa genes, the remaining 131 genes were associated with a 0.41-year younger age at diagnosis, although this did not reach statistical significance (95% CI=-0.84 to 0.03, P=.07). A larger effect was observed for the 24 candidate genes, with carriers having a 1.06-year younger age at diagnosis (95% CI=-1.65 to -0.48, $P=3.71 \times 10^{-4}$), which reduced to a 0.55-year younger age at diagnosis after removing *BRCA2*, *PALB2*, and *ATM* (95% CI=-1.21 to 0.11, P=.10; **Supplementary Figure 6C**). P/LP/D alleles in the BER pathway were nominally associated with a younger age at diagnosis by 0.74 years (95% CI=-1.43 to -0.06, P=.03), although this was not statistically significant after correcting for multiple testing (**Supplementary Table 6**). Associations with age at diagnosis did not statistically

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significantly differ in analyses stratified by disease aggressiveness (**Supplementary Tables 5-7**).

Stratified Analyses

BRCA2, *PALB2*, *ATM*, and the aggregate 155 DNA repair genes and 24 candidate genes were further assessed in stratified analyses. We observed larger effects of P/LP/D *BRCA2* alleles on aggressive PCa, PCa death, and metastatic disease among men diagnosed <60 versus ≥60 years of age; however, these results did not statistically significantly differ between age strata (**Supplementary Table 8**). The effects of *PALB2*, *ATM*, the aggregate 155 DNA repair genes, and the aggregate 24 candidate genes did not statistically significantly differ by age at diagnosis. No statistically significant differences were observed in analyses stratified by PCa family history (**Supplementary Table 9**).

Risk associated with *BRCA2* statistically significantly differed by country (P=.04; **Supplementary Table 10**), with the strongest associations with aggressive disease observed in men from the United Kingdom (OR=10.11, 95% CI=2.23 to 45.76, $P=1.22x10^{-4}$), followed by Australia (OR=5.60, 95% CI=1.65 to 19.05, P=.002), the United States (OR=2.84, 95% CI=0.85 to 9.41, P=.07), and Sweden (OR=1.91, 95% CI=0.76 to 4.81, P=.16), with no evidence of association in Finnish men (OR=0.69, 95% CI=0.15 to 3.14, P=.62). Differences were also observed for the aggregate 24 candidate genes (P=.01), with the strongest associations with aggressive disease observed in men from the United Kingdom (OR=2.24, 95% CI=1.50 to 3.35, $P=4.92x10^{-5}$), followed by Finland (OR=1.88, 95% CI=1.17-3.02, P=.008), with statistically non-significant

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effects observed in men from Sweden, the United States, and Australia (ORs<1.30). These differences remained statistically significant after excluding *BRCA2*, *PALB2*, and *ATM* (P=.03; **Supplementary Table 10**), indicating the potential importance of the remaining 21 genes for certain populations.

Among non-metastatic aggressive cases, the highest *BRCA2* carrier frequency was observed in those with T1/T2 and Gleason ≥8 (4.7%, P=3.65x10^{-7}), followed by those with T3/T4 and Gleason ≥8 (2.5%, P=3.55x10^{-5}), T3/T4 and Gleason <8 (1.9%, P=.33), and T1/T2 and Gleason <8 tumors (1.6%, P=.20) relative to non-aggressive cases (0.8%) (**Supplementary Table 11**). The aggregate 24 candidate PCa genes also had the highest carrier frequency in non-metastatic aggressive cases with T1/T2 and Gleason ≥8 tumors (15.8%, P=.01), followed by cases with T3/T4 and Gleason <8 (14.3%, P=.11), T3/T4 and Gleason ≥8 (14.0%, P=.03), and T1/T2 and Gleason <8 tumors (11.6%, P=.44) (**Supplementary Table 11**).

DISCUSSION

In this international case-case investigation of 5,545 men with PCa, we investigated whether rare P/LP/D variants in 155 DNA repair genes differentiate risk of aggressive versus non-aggressive disease. *BRCA2* and *PALB2* were associated with the greatest risk, with P/LP/D *BRCA2* carriers having 3.2-fold increased risk of aggressive PCa and P/LP/D *PALB2* carriers having 6.3-fold increased risk of aggressive PCa. *ATM* had nominal evidence of association, with P/LP/D *ATM* carriers having 1.9-fold increased risk of aggressive PCa. Our candidate set of 24 DNA repair genes had higher aggregate carrier frequencies in aggressive (14.2%) and metastatic (15.4%) than

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non-aggressive (10.6%) PCa cases; however, these differences were largely driven by *BRCA2*, *PALB2*, and *ATM*.

Although *PALB2* has been suspected to be a PCa susceptibility gene, due to the rarity of pathogenic variants in this gene, little statistical evidence has supported an association between *PALB2* and PCa[35]. *PALB2* is an important biological link between *BRCA1* and *BRCA2* needed for homologous recombination repair after double-strand breaks[36], and rare pathogenic *PALB2* variants have been reported to increase risk of breast, ovarian, and pancreatic cancer[37-39]. One investigation reported marginal evidence of pathogenic *PALB2* variants being associated with a 3.5-fold increased risk (95% CI=0.7-10.3, P=.05) of metastatic PCa when compared to cancer-free controls in the Exome Aggregation Consortium[16]. A recent study found that *PALB2* was an important risk factor for overall and aggressive PCa in African American and Ugandan men, in addition to *BRCA2* and *ATM*[20], which is of particular importance given that men of African descent have increased risk of aggressive PCa[40]. Other studies have also reported *ATM* to be associated with increased risk of aggressive PCa[16, 17], providing external support for the nominal *ATM* associations we observed.

The associations we identified between *BRCA2* and increased risk of aggressive PCa are consistent with previous studies[16, 17, 19, 41]. We identified heterogeneous *BRCA2* effects between populations, with larger effects seen in men from the United Kingdom and null effects in Finnish men, consistent with previous null findings in this population[42]. While we report fairly similar carrier frequencies among metastatic cases for 20 DNA repair genes investigated by Pritchard et al.[16] (**Supplementary Table 12**),

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BRCA2 is a notable exception, being substantially more common among metastatic cases in this previous report (5.35%) than the current study (1.93%), and less common in TCGA primary PCa cases (0.20%), used as their comparison group, than our non-aggressive cases (0.83%). Another recent study[17] reported a similar *BRCA2* carrier frequency among high-grade PCa cases (2.55%) as our aggressive cases (2.49%); however, they reported a lower frequency among low-grade cases (0.20%) than our non-aggressive cases (0.83%). “Winner’s curse” may contribute to the larger *BRCA2* effect observed in these previous studies given their smaller sample sizes[43]. Differences in carrier frequencies and/or effect sizes between studies may also be attributed to different compositions of aggressive and non-aggressive comparison groups.

We observed suggestive evidence of associations between the MMR pathway, which is associated with Lynch syndrome[44], and Lynch syndrome genes *MLH1* and *MSH2* contributing to risk of aggressive PCa. While additional studies are needed to validate these findings, MMR variant carriers have been reported to have increased PCa risk, higher Gleason scores, and younger PCa diagnoses[45], and loss of MSH2 protein has been observed among high-grade primary PCa tumors[46].

The aggregate 24 candidate PCa genes were associated with younger age at PCa diagnosis, with some residual effect remaining after excluding the strongest risk-increasing genes, *BRCA2*, *PALB2*, and *ATM*. While gene-based associations with age at diagnosis were not statistically significant after correcting for multiple testing, our nominal association between *BRCA2* and younger age at diagnosis is consistent with previous studies[47, 48]. We also observed suggestive evidence for greater risk of

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aggressive PCa in *BRCA2* carriers with a younger versus older age at diagnosis, which builds on previous reports of overall PCa risk being greater in younger than older *BRCA2* carriers[49]. Younger disease onset is typically attributed to stronger genetic predisposition, which may be partially attributable to P/LP/D *BRCA2* variants for PCa.

Although our investigation represents the largest DNA repair gene sequencing study of PCa to date, the study was still underpowered to detect statistically significant associations in single variant and gene burden testing. For example, to detect an OR of 2.0 with 90% power and a 0.25% carrier frequency in non-aggressive cases, over 25,000 total cases would be needed. Until such samples are available, it will be difficult to nominate specific genes for personalized risk prediction of PCa and/or aggressive disease based on statistical evidence. This is supported by our observation that a multi-gene burden test of candidate DNA repair genes was no longer predictive of aggressive disease after removing the top three genes, *BRCA2*, *PALB2*, and *ATM* (OR=1.14, 95% CI=0.94 to 1.37, P=.18), with further risk reduction observed when removing the top seven genes (OR=0.94, 95% CI=0.74 to 1.19, P=.59 for the remaining 17 genes). A larger sample will also be necessary to identify genetic factors that distinguish subgroups of aggressive disease. Further, among our top findings, we observed association differences by country; while this can likely be partly attributed to genetic differences, it is possible that differences in the composition of aggressive and non-aggressive cases by country (**Table 1**) also contributed to these differences.

Our results suggest that PCa risk conveyed by DNA repair genes is largely driven by rare P/LP/D alleles within *BRCA2*, *PALB2*, and *ATM*, with suggestive evidence that *MLH1*, *CHEK2*, *MUTYH*, and *MSH2* are also associated with increased

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risk of aggressive and metastatic disease. It was recently recommended that *BRCA2* carrier status be factored into determining the initial age of PCa screening and intervals of subsequent screenings, while *BRCA2* and *ATM* be factored into high-risk and advanced PCa disease management[50]. Our findings support the importance of these genes as well as *PALB2* in both screening and disease management considerations. The decision to undergo genetic testing in men without PCa is typically based on family history; however, it was recently shown that men with PCa who do not have a family history carry P/LP alleles[51]. Universal genetic testing to tailor PSA screening will require additional research and support of the clinical availability of such genetic testing. While the modest risk conveyed by P/LP/D alleles within 24 candidate DNA repair genes provides important information regarding disease etiology, particularly given the sparsity of known risk factors for aggressive PCa beyond obesity[52], genes with larger effects, such as *BRCA2*, *PALB2*, and *ATM*, should be prioritized in future genetic risk prediction testing for PCa. In addition to the need to better understand the relative risks of each of these genes in aggressive and non-aggressive disease compared to cancer-free controls, research is needed to understand the role of rare coding variation in genes that function outside of DNA repair in overall and aggressive PCa.

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DATA AVAILABILITY STATEMENT

Whole-exome sequencing data along with the clinical status of each participant in this investigation is available through the database of genotypes and phenotypes (dbGaP, accession number: phs001524.v1.p1).

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TABLES

Table 1. Clinical characteristics of study participants by study (N=5,545).

Characteristic	Overall	Australia			Finland	United States			United Kingdom	Sweden			
		APCS	MCCS	PCFS	ATBC	CPS-II	MEC	PLCO	ICR	CAPS	PROCAP	STHM1	STHM2
Total No.	5,545	219	413	442	916	360	203	394	1,061	690	248	205	394
Aggressive, No.	2770	219	114	198	466	169	106	190	530	452	31	67	228
Age at Dx, Mean (SD)	66.1 (8.8)	64.9 (5.8)	70.6 (7.4)	57.9 (8.4)	70.4 (6.1)	73.3 (6.9)	70.7 (9.0)	70.1 (6.4)	57.1 (5.2)	67.3 (7.6)	63.2 (5.2)	69.6 (7.6)	70.0 (8.7)
Family History, No. (%) [a]													
Yes	331 (11.9)	0 (0)	15 (13.2)	32 (16.2)	27 (5.8)	22 (13.0)	6 (5.7)	18 (9.5)	111 (20.9)	79 (17.5)	10 (32.3)	11 (16.4)	0 (0)
No	1776 (64.1)	2 (0.9)	67 (58.8)	137 (69.2)	380 (81.5)	147 (87.0)	90 (84.9)	168 (88.4)	351 (66.2)	373 (82.5)	6 (19.4)	55 (82.1)	0 (0)
Death Due to PCa, No. (%) [a]													
Yes	2052 (74.1)	21 (9.6)	89 (78.1)	156 (78.8)	386 (82.8)	127 (75.1)	61 (57.5)	117 (61.6)	530 (100.0)	362 (80.1)	31 (100.0)	66 (98.5)	106 (46.5)
No	311 (11.2)	0 (0)	0 (0)	0 (0)	80 (17.2)	0 (0)	18 (17.0)	0 (0)	0 (0)	90 (19.9)	0 (0)	1 (1.5)	122 (53.5)
Metastatic Disease, No. (%) [a]													
Yes	467 (16.9)	4 (1.8)	17 (14.9)	10 (5.1)	186 (39.9)	0 (0)	29 (27.4)	47 (24.7)	174 (32.8)	0 (0)	0 (0)	0 (0)	0 (0)
No	663 (23.9)	3 (1.4)	0 (0)	1 (0.5)	234 (50.2)	0 (0)	74 (69.8)	131 (68.9)	220 (41.5)	0 (0)	0 (0)	0 (0)	0 (0)
Stage, No. (%) [a]													
1	410 (14.8)	14 (6.4)	59 (51.8)	109 (55.1)	63 (13.5)	77 (45.6)	31 (29.2)	25 (13.2)	16 (3.0)	0 (0)	4 (12.9)	12 (17.9)	0 (0)
2	367 (13.2)	0 (0)	2 (1.8)	10 (5.1)	109 (23.4)	0 (0)	0 (0)	36 (18.9)	66 (12.5)	71 (15.7)	27 (87.1)	15 (22.4)	31 (13.6)
3	1277 (46.1)	194 (88.6)	34 (29.8)	67 (33.8)	91 (19.5)	92 (54.4)	43 (40.6)	77 (40.5)	164 (30.9)	315 (69.7)	0 (0)	33 (49.3)	167 (73.2)
4	654 (23.6)	11 (5.0)	19 (16.7)	12 (6.1)	203 (43.6)	0 (0)	29 (27.4)	52 (27.4)	230 (43.4)	64 (14.2)	0 (0)	4 (6.0)	30 (13.2)
Gleason Score, No (%) [a]													

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≤6	197 (7.1)	0 (0)	4 (3.5)	1 (0.5)	109 (23.4)	22 (13.0)	4 (3.8)	28 (14.7)	9 (1.7)	18 (4.0)	0 (0)	0 (0)	2 (0.9)
7	490 (17.7)	2 (0.9)	28 (24.6)	80 (40.4)	88 (18.9)	36 (21.3)	27 (25.5)	38 (20.0)	16 (3.0)	133 (29.4)	9 (29.0)	0 (0)	33 (14.5)
8–10	1862 (67.2)	217 (99.1)	70 (61.4)	115 (58.1)	134 (28.8)	82 (48.5)	67 (63.2)	114 (60.0)	484 (91.3)	299 (66.2)	22 (71.0)	67 (100.0)	191 (83.8)
Non-Aggressive, No.	2775	0	299	244	450	191	97	204	531	238	217	138	166
Age at Dx, Mean (SD)	67.5 (7.0)	--	71.5 (6.7)	65.0 (6.5)	71.9 (4.7)	68.3 (5.0)	67.8 (6.8)	68.4 (5.5)	62.6 (6.0)	66.9 (7.4)	63.7 (5.1)	73.4 (3.8)	67.0 (7.9)
Family History, No. (%) [a]													
Yes	467 (16.8)	--	47 (15.7)	56 (23.0)	17 (3.8)	53 (27.7)	14 (14.4)	20 (9.8)	123 (23.2)	50 (21.0)	58 (26.7)	29 (21.0)	0 (0)
No	1816 (65.4)	--	199 (66.6)	137 (56.1)	385 (85.6)	138 (72.3)	80 (82.5)	181 (88.7)	350 (65.9)	188 (79.0)	49 (22.6)	109 (79.0)	0 (0)
Stage, No. (%)													
1	2383 (85.9)	--	262 (87.6)	198 (81.1)	267 (59.3)	191 (100.0)	97 (100.0)	78 (38.2)	531 (100.0)	238 (100.0)	217 (100.0)	138 (100.0)	166 (100.0)
2	392 (14.1)	--	37 (12.4)	46 (18.9)	183 (40.7)	0 (0)	0 (0)	26 (61.8)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Gleason Score ≤6, No. (%) [a]	2773 (99.9)	--	299 (100.0)	244 (100.0)	450 (100.0)	191 (100.0)	97 (100.0)	204 (100.0)	531 (100.0)	238 (100.0)	217 (100.0)	136 (98.6)	166 (100.0)

[a] Numbers do not sum to the total sample size due to missing data.

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Table 2. Association results for top 15 DNA repair genes and 24 candidate PCa genes.

Gene	Rank/ 81[a]	Chr	No. of Var	Carrier Frequencies			Aggressive v Non-Aggressive			Metastatic v Non-Aggressive		
				Non-Agg	Agg	M1	OR (95% CI)	P[b]	P.adj	OR (95% CI)	P[b]	P.adj
Top 15 Genes												
BRCA2 [c]	1	13	59	0.00829	0.02491	0.01927	3.19 (1.94–5.25)	8.58×10^{-7} [d]	6.95×10^{-5}	2.88 (1.22–6.83)	.02	.25
PALB2 [c]	2	16	16	0.00108	0.00650	0.01071	6.31 (1.83–21.68)	4.79×10^{-4} [d]	.02	7.71 (1.62–36.72)	.009	.19
TP53BP1	3	15	2	0.00360	0.00072	0	0.18 (0.04–0.86)	.01	.36	—	.07	.51
ATM [c]	4	11	47	0.00757	0.01552	0.02141	1.88 (1.10–3.22)	.02	.38	2.71 (1.19–6.2)	.02	.25
GEN1 [c]	5	2	8	0.00396	0.00072	0	0.22 (0.05–1.02)	.03	.42	—	.23	.64
ALKBH3	6	11	5	0.00865	0.00469	0	0.50 (0.25–1.01)	.049	.53	—	.01	.21
APLF	7	2	5	0.00216	0.00036	0	0.17 (0.02–1.44)	.05	.53	—	.06	.51
RECQL	8	12	10	0.00757	0.00433	0	0.52 (0.25–1.07)	.07	.53	—	.005	.19
FANCG	9	9	3	0.00180	0.00036	0	0.17 (0.02–1.55)	.07	.53	NA	NA	NA
MLH1 [c]	10	3	11	0.00865	0.01227	0.01285	1.62 (0.94–2.82)	.08	.53	2.15 (0.8–5.75)	.15	.63
FANCM	11	14	11	0.00829	0.01300	0.01071	1.61 (0.93–2.79)	.08	.53	1.1 (0.39–3.13)	.85	.94
DCLRE1C	12	10	4	0.00180	0.00036	0	0.20 (0.02–1.72)	.09	.53	NA	NA	NA
MDC1	13	6	2	0.00180	0.00036	0	0.20 (0.02–1.78)	.10	.53	—	.31	.72
EXO1	14	1	6	0.00577	0.00975	0.00857	1.70 (0.90–3.23)	.10	.53	1.43 (0.46–4.47)	.56	.87
FANCD2	15	3	4	0.00216	0.00072	0	0.28 (0.06–1.42)	.10	.53	—	.12	.61
Remaining candidate PCa DNA Repair Genes												
BRIP1 [c]	16	17	7	0.00252	0.00072	0.00214	0.32 (0.06–1.59)	.13	.67	0.93 (0.1–8.66)	.95	.97
CHEK2 [c]	17	22	16	0.01405	0.01913	0.02141	1.38 (0.89–2.14)	.14	.68	1.63 (0.76–3.51)	.23	.64
MUTYH [c]	28	1	10	0.01333	0.01661	0.01713	1.27 (0.81–1.99)	.30	.81	1.51 (0.67–3.4)	.34	.73
MSH2 [c]	31	2	9	0.00108	0.00217	0.00214	2.06 (0.46–9.28)	.33	.81	NA	NA	NA
BRCA1 [c]	37	17	15	0.00252	0.00361	0.00428	1.55 (0.56–4.29)	.39	.85	2.11 (0.37–12.21)	.42	.80
MSH6 [c]	39	2	8	0.00396	0.00578	0.00428	1.36 (0.62–2.98)	.44	.89	1.19 (0.24–5.86)	.84	.94
MRE11A [c]	41	11	8	0.00216	0.00144	0	0.61 (0.17–2.25)	.45	.89	—	.19	.64

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ATR [c]	48	3	9	0.01766	0.01552	0.01285	0.87 (0.57–1.34)	.53	.90	0.81 (0.33–1.99)	.64	.90
NBN [c]	50	8	6	0.00180	0.00144	0.00642	0.69 (0.18–2.60)	.58	.93	4.11 (0.83–20.3)	.10	.59
WRN [c]	52	8	7	0.00396	0.00542	0.00214	1.22 (0.55–2.74)	.62	.96	0.48 (0.06–3.95)	.45	.80
PMS2 [c]	62	7	7	0.00144	0.00108	0.00214	0.82 (0.17–3.96)	.81	.98	NA	NA	NA
FANCL [c]	67	2	7	0.00973	0.00903	0.01071	1.05 (0.60–1.85)	.87	.98	1.21 (0.44–3.35)	.72	.92
XPC [c]	70	3	3	0.00108	0.00108	0.00214	1.14 (0.22–5.85)	.88	.98	NA	NA	NA
FAM175A [c]	79	4	8	0.00180	0.00181	0	0.97 (0.26–3.60)	.97	.98	NA	NA	NA
ERCC2 [c]	80	19	13	0.00324	0.00361	0.00642	0.98 (0.39–2.46)	.97	.98	2.48 (0.59–10.46)	.24	.64
RAD51C [c]	--	17	4	0.00036	0.00144	0	NA	NA	NA	NA	NA	NA
RAD51D [c]	--	17	2	0.00036	0.00072	0.00214	NA	NA	NA	NA	NA	NA
SLX4 [c]	--	16	5	0.00108	0.00072	0	NA	NA	NA	NA	NA	NA
XRCC2 [c]	--	7	4	0.00036	0.00108	0	NA	NA	NA	NA	NA	NA

Abbreviations: “#Var”: Number of P/LP/D variants identified. “Non-Agg”: Non-aggressive cases. “Agg”: Aggressive cases. “M1”: Metastatic cases. “P.adj”: Benjamini-Hochberg adjusted P-values, calculated using an alpha 0.05. “NA”: Test not performed because the minor allele count was five or less between non-aggressive and metastatic cases. “—": Effect could not be calculated due to no alleles being present in metastatic cases.

[a] Ranking is based on the P-values for Aggressive versus Non-Aggressive PCa

[b] P-values are calculated using the likelihood-ratio test. All tests of statistical significance are two-sided.

[c] Subset of 24 literature-curated candidate PCa genes

[d] Statistically significant

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FIGURE LEGENDS

Figure 1. Distribution of 289 rare pathogenic/deleterious variants among 24 candidate PCa DNA repair genes. Genes (No. of variants) are shown.

Figure 2. Carrier frequencies and effects of candidate PCa DNA repair genes (DRG). Carrier frequencies (A) and effects (B) of candidate PCa genes by disease aggressiveness (*RAD51C*, *RAD51D*, *SLX4*, and *XRCC2* were not evaluated in gene-based tests, as our sample had ≤5 carriers). Aggregate carrier frequencies (C) and aggregate effects (D) of DNA repair genes, sequentially removing the strongest genes. Left panels aggregate all DNA repair genes, including and excluding the 24 candidate PCa DRG genes. Right panels aggregate the 24 candidate PCa DRG genes, sequentially removing the seven genes with the strongest risk-increasing effects. The remaining PCa DRG genes had no aggregate effect on aggressive disease (excluding top three genes: *BRCA2*, *PALB2*, and *ATM*, P=.18; excluding top seven genes: *BRCA2*, *PALB2*, *ATM*, *MLH1*, *CHEK2*, *MUTYH*, and *MSH2*, P=.59).

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Figure 1--FINAL

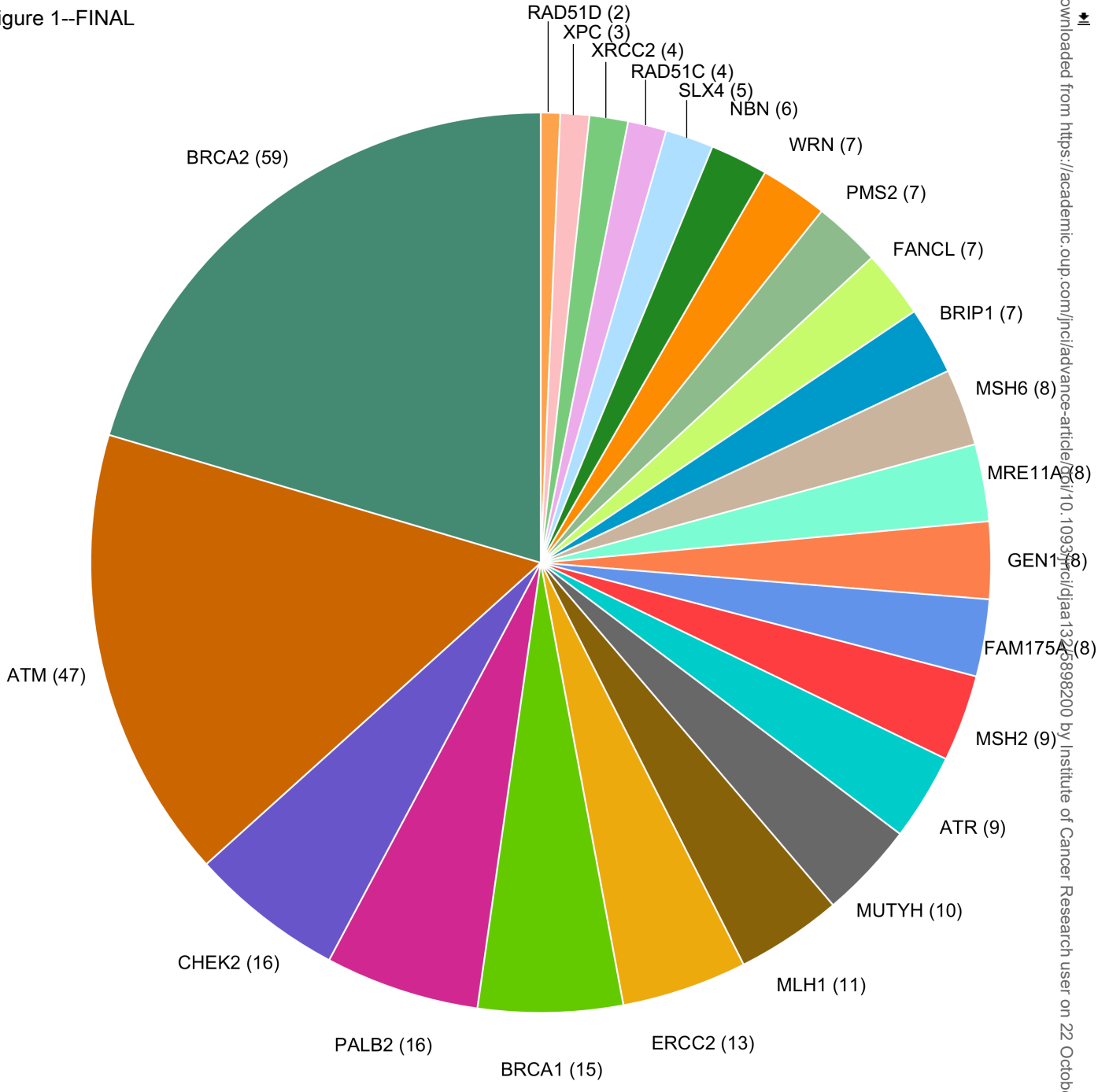

Figure 1

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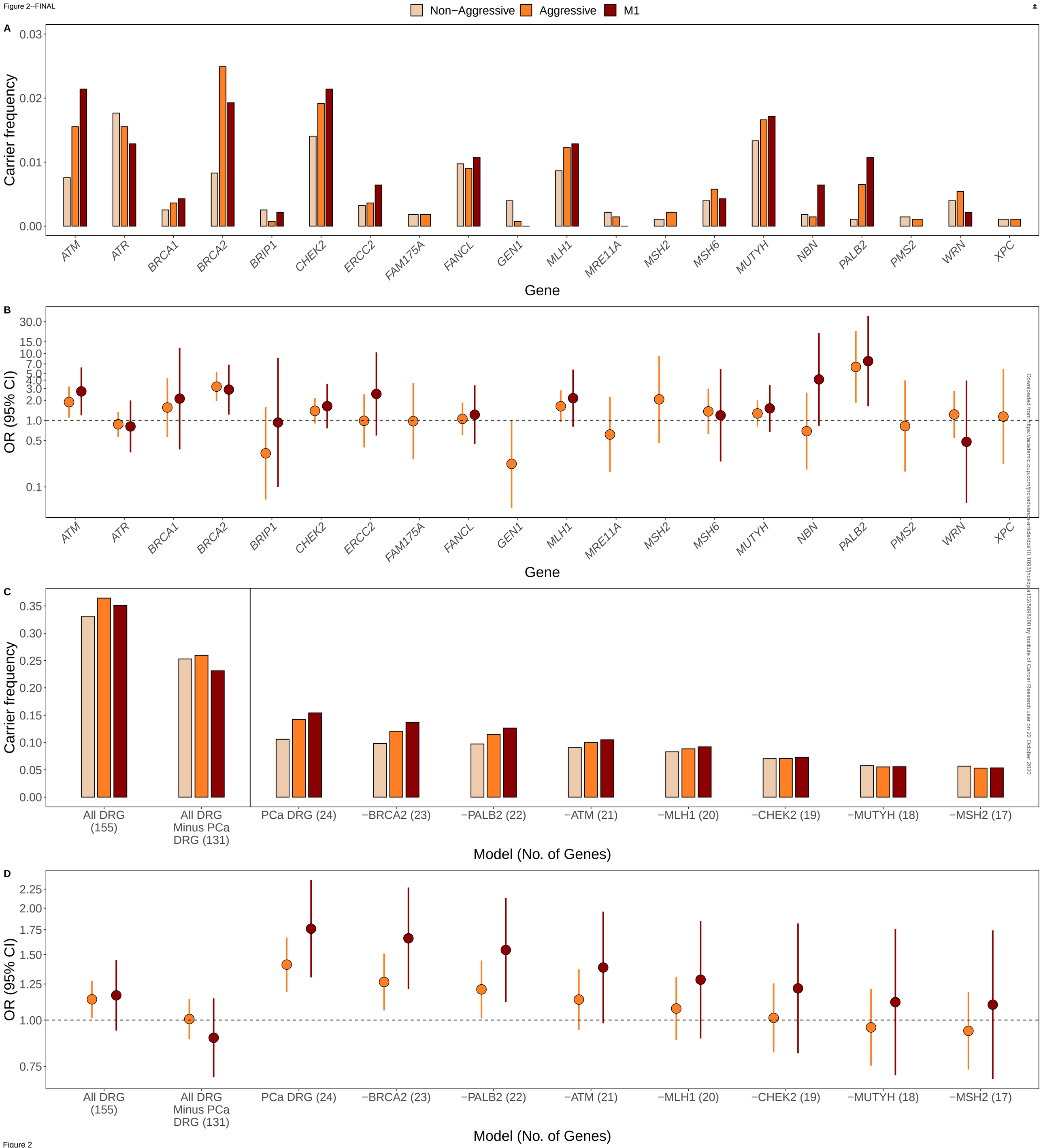

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