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Laboratory-Prostate cancer

Germline variants in early and late-onset Brazilian prostate cancer patients

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Abstract

Background: The median age for Prostate Cancer (PCa) diagnosis is 66 years, but 10% are diagnosed before 55 years. Studies on early-onset PCa remain both limited and controversial. This investigation sought to identify and characterize germline variants within Brazilian PCa patients classified as either early or later onset disease.

Methods: Peripheral blood DNA from 71 PCa patients: 18 younger (≤ 55 years) and 53 older (≥ 60 years) was used for Targeted DNA sequencing of 20 genes linked to DNA damage response, transcriptional regulation, cell cycle, and epigenetic control. Subsequent genetic variant identification was performed and variant functional impacts were analyzed with in silico prediction.

Results: A higher frequency of variants in the *BRCA2* and *KMT2C* genes across both age groups. *KMT2C* has been linked to the epigenetic dysregulation observed during disease progression in PCa. We present the first instance of *KMT2C* mutation within the blood of Brazilian PCa patients. Furthermore, out of the recognized variants within the *KMT2C* gene, 7 were designated as deleterious. Thirteen deleterious variants were exclusively detected in the younger group, while the older group exhibited 37 variants. Within these findings, 4 novel variants emerged, including 1 designated as pathogenic.

Conclusions: Our findings contribute to a deeper understanding of the genetic factors associated with PCa susceptibility in different age groups, especially among the Brazilian population. This is the first investigation to explore germline variants specifically in younger Brazilian PCa patients, with high relevance given the genetic diversity of the population in Brazil. Additionally, our work presents evidence of functionally deleterious germline variants within the *KMT2C* gene among Brazilian PCa patients. The identification of novel and functionally significant variants in the *KMT2C* gene emphasizes its potential role in PCa development and warrants further investigation.

Keywords: Prostate cancer; Germline variants; DNA sequencing; Early-onset; Late-onset

1. Introduction

The burden of cancer incidence and mortality is rapidly growing worldwide, as a result of population aging and

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growth as well as changes in the prevalence and distribution of the main risk factors for cancer [1].

Prostate cancer (PCa) is the most frequent noncutaneous cancer diagnosed [2], and the fifth leading cause of cancer death in men worldwide (https://gco.iarc.fr/). In Brazil, 71.730 new cases and 16.300 deaths by PCa were estimated for 2023 to 2025 [3]. Risk factors for PCa include age, family cancer history, African ancestry, and genetic factors [4]. A Norwegian twin pairs study suggested that up to 57% of PCa can be attributed to genetic risk factors [5]. Consistent reports have identified germline mutations in the genes *BRCA1, BRCA2, MMR, HOXB13, and CHEK2,* as conferring moderate risks, with some leading to a more aggressive disease behavior [6].

Early onset PCa exhibits distinct clinical behavior, characterized by a worse prognosis in advanced cases, implying the presence of a unique clinical subtype within this patient subset. While most PCa cases are diagnosed at the median age of 66 years, 10% occur before the age of 55 [7]. Despite the importance of identifying rare alleles that may contribute to the development of aggressive disease in younger men, reports remain limited, particularly in Brazil. The varying rates of PCa incidence, progression, and genetic factors among diverse ethnicities and geographic regions suggest potential contributions to country-specific patterns of disease evolution and genetic susceptibility.

The Brazilian population's distinct genetic composition, resulting from the admixture of different ancestral backgrounds, might impact disease vulnerability and genetic predisposition to PCa. This study represents the first examination of germline variants in Brazilian PCa patients aged 55 or below, which, coupled with the Brazilian population's mixed genetic heritage, underscores the significance of this study.

In this work, we selected 20 genes involved in DNA Damage Response, DNA repair genes (DRGs), transcriptional regulation, cell cycle, and epigenetic regulation associated with PCa progression, which were analyzed in a cohort of PCa patients stratified by age of diagnosis. This approach allowed the identification of exclusive and deleteriousness germline variants in younger and older Brazilian patients, revealing mutations in *KMT2C* that have not previously been found in the blood of PCa patients.

2. Materials and methods

2.1. Patients and sample collection

This study included 71 men with primary PCa. Peripheral blood samples were collected in 2 Brazilian hospitals and all participants provided informed consent. Comprehensive clinicopathological data were obtained from medical records (Supplementary Table 1). Samples were collected prior to any specific cancer treatment, and genomic DNA isolation was conducted using the DNeasy Blood & Tissue Kit (Qiagen), following the manufacturer's instructions.

2.2. Targeted DNA sequencing

Our study utilized Targeted DNA Sequencing to capture all exonic regions, as well as upstream (1000 bp) and downstream regions (200 bp) of each gene. An Illumina NovaSeq 600, in paired-end mode, was used to sequence the libraries constructed with MyBaits Custom DNA Sequencing kits (Daicel Arbor Biosciences). Germline variant analysis was performed according to the Genome Analysis Toolkit (GATK) Best Practices (https://gatk.broadinstitute.org/hc/en-us) for single nucleotide polymorphisms (SNPs). Sequences were aligned against the human reference genome GRCh38 (https://hgdownload.soe.ucsc.edu/) using the Burrows-Wheeler Alignment (BWA) mem tool (version 0.7.17-r1188) and the variant calling was performed using the Haplotype Caller tool. We used SnpEff (https://sourceforge.net/projects/snpeff/) and SnpSift (http://pcingola.github.io/SnpEff/) for variant annotation. Variants with DP<50 and those identified in any of the publicly accessible databases for healthy individuals—both global databases (1000Gp3, ExAC, and gnomAD) and the Brazilian database (ABraOM)—were excluded from our analysis. Our sequencing analyses yielded a mean coverage depth (DP) of 2700x per sample with 92% of targeted regions achieving a coverage depth of $\geq$ 50x.

2.3. Variation classification

Variants were classified according to the American College of Medical Genetics and Genomics (ACMG) guidelines using VarSome (https://varsome.com/). The pathogenicity scores were assessed with in silico prediction tools: CADD [8], PolyPhen-2 [9], and SIFT [10]. To identify exclusive variants in each age group with any pathogenicity grade, we utilized the tools mentioned above, which resulted in variants with at least one of these criteria: (CADD $\geq$ 15), PolyPhen-2 (probably/possibly_damaging), SIFT (deleterious), and ACMG classification (variant of uncertain significance [VUS]/likely pathogenic [LP]/pathogenic [P]).

2.4. Statistical analysis

All statistical analyses were performed using the R (version 4.1.2) software. The correlation between the qualitative variables was analyzed using Fisher's Exact Test. The Kaplan-Meier method was used for survival analysis (log-rank test). P values < 0.05 were considered significant.

3. Results

3.1. Tumor classification and age-related trends

Of the 71 men recruited in this study, 18 were aged $\leq$ 55 years (younger group) and 53 were $\geq$ 60 years old (older group). At diagnosis, the younger group exhibited a lower, albeit nonsignificant median total PSA value as compared to

the older group ($P = 0.2873$). The ISUP 2 was the prevailing classification. ISUP 1 was the most frequent in the younger group, while in the older group, it was ISUP 2, but these differences were not significant ($P = 0.6808$). Younger and older patients predominantly presented with palpable tumors confined solely to the prostate (tumor stage T2), with no significant difference noted based on age ($P = 0.5638$). Collecting family history (FH) from electronic medical records yielded data from 12 younger individuals and 31 older individuals. Among the younger patients, 75% had positive cancer FH, while within the older group, FH was observed in 58% of cases ($P = 0.3152$). Despite the constraint imposed by the limited sample size, our study points toward a more pronounced hereditary factor within the younger cohort. When evaluating the type of malignancy in FH, we observed that PCa accounted for half of the cancer cases (Supplementary Table 2).

3.2. Variants and mutation distribution

Following alignment, pre-processing, and variant calling, a total of 261 nonsynonymous variants were identified across the 20 analyzed genes, with 122 in the younger group and 139 in the older group. After filtering for Single nucleotide variants (SNVs) with DP ≥ 50, there remained 81 variants (22 in the younger group and 59 in the older group) were identified in 15 of the 20 genes from 46 of the 71 samples (Fig. 1**).** As expected, *BRCA2* exhibited the highest alteration rate, accounting for 23% of cases. Among the 11 patients with *BRCA2* variants, distinct variants were identified: 10 were missense and 1 was nonsense. Notably, 3 of these *BRCA2* variants were found in 2 patients each: rs28897701, rs11571831, and rs45574331. Intriguingly, *KMT2C* emerged as the second most frequently mutated gene (19%), with 2 patients bearing the variant rs111826855, and 1 patient harboring 2 variants, namely rs547763902 and rs567984906. A schematic representation of the most mutated genes (*BRCA2* and *KMT2C*) and their functional domains on the protein are shown in Figure 2. In contrast, *TP53* and *PARP1* exhibited the least alteration, with variants detected in only 2% of the patients. Moreover, none of the patients displayed germline variants in the *CHEK2*, *HOXB13, FOXA1,* or *SPOP* genes. The missense variants rs35001569 and rs63750449, located in the same codon of the *MLH1* gene, recurred most frequently appearing in 3 cases: 2 from the younger group and 1 from the older group. Both *MLH1* variants were predicted as damaging/pathogenic according to the silico prediction tools.

3.3. Age-dependent SNV patterns

To determine whether there were any potentially unique SNVs associated with the age groups, we performed an analysis of SNVs with a DP ≥ 50, using the VENNY 2.1 tool (https://bioinfogp.cnb.csic.es/tools/venny/). Using *HGVs coding* we found 17 exclusive variants in the younger group (Supplementary Table 3), 54 exclusive variants in the older group (Supplementary Table 4), and 5 variants common to both groups (Supplementary Table 5) (Fig. 3). The 17 exclusive variants found solely in the younger group are

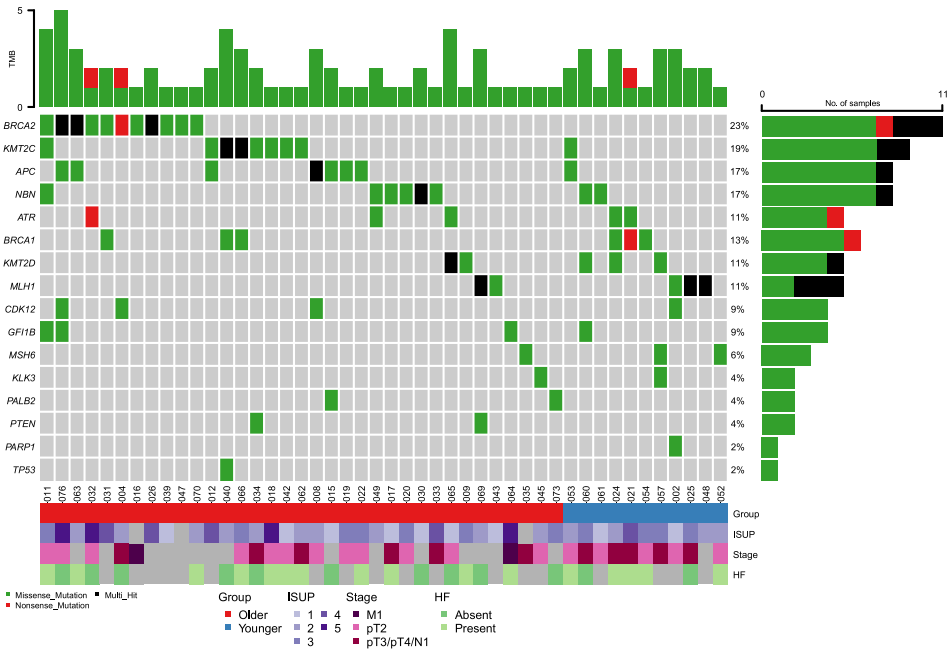

Fig. 1. Oncoplot displaying the mutated genes in blood samples of the younger (blue) and older (red) PCa patients. The oncoplot provided an overview of mutation ratios, mutation types, and clinical features for each patient. The right oncoplot shows the ratio of each mutated gene, and the bottom oncoplot shows the sample ID and the clinical features of the corresponding patient. Variants annotated as Multi_Hit are those genes that are mutated more than once in the same sample. HF = family history cancer; ISUP = ISUP grade; Stage = tumoral stage. (younger, $n = 18$ and older individuals, $n = 53$).

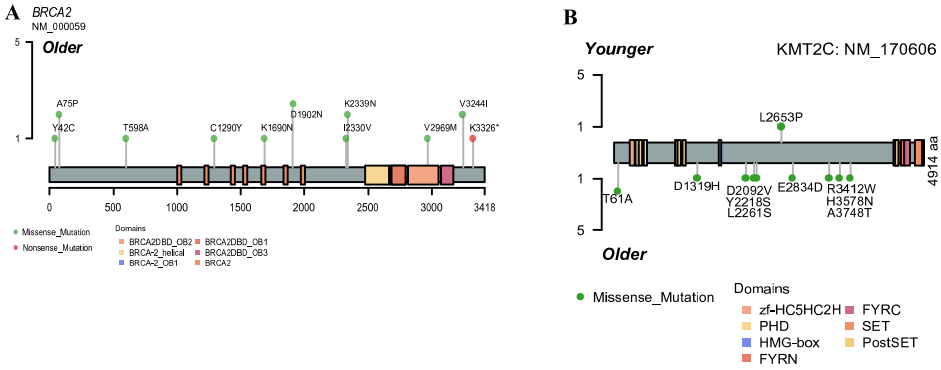

Fig. 2. *BRCA2* and *KMT2C* protein: location of mutations and domains in the protein encoded by (A) *BRCA2* and (B) *KMT2C* genes. The variants found in our cohort are shown by a lollipop plot, with the mutation type indicated by color. Up: mutations in the younger group and down: mutations in the older group.

distributed across 11 genes detected in 9 samples from the 18 cases in the younger age group. Notably, nearly 94% of these were missense variants, with *ATR* (33%, 3/9) and *KMT2D* (33%, 3/9) genes exhibiting higher rates of alteration within the patient subset. In parallel, the 54 variants exclusively identified within the older group were distributed across 15 genes from 35 of the 53 samples. Once again, the prevalent variants were missense mutations. Within this group, *BRCA2* (31%, 11/35) and *KMT2C* (23%, 8/35) were the genes displaying the highest rates of alteration and the greatest number of variants. Exclusive multihit variants in the older group were identified within *APC, BRCA2, KMT2C/D,* and *NBN,* genes (Supplementary Fig. 1). The younger group showed a smaller number of variants compared to the older group, possibly attributed to the limited sample size of younger patients. Nevertheless, the significance of these variants lies in their exclusivity to the younger age group, warranting further attention.

3.4. Cataloged variants in dbSNP database

Within the set of 17 variants exclusive to the young group, 16 are documented in dbSNP, while 1 was classified as a novel variant. Among the 54 variants exclusive to the older age group, 51 were present in dbSNP, while 3 were novel. Notably, all 5 variants common to both groups had already been described in dbSNP (Fig. 3). We then subjected the variants to filtering using in silico online tools, retaining the variants that fulfilled at least one of the criteria mentioned (see Fig. 3, step 4). There were 13 of 16 dbSNP-exclusive variants from the young group successfully passed through these applied filters. In essence, among 16 exclusive variants within this group, 13 of 16 (81%) were predicted as damaging/pathogenic in at least one of these in silico prediction tools. (Table 1**).** For example, the pathogenic variant (rs80356923) in the *BRCA1* gene, is known for its clinical significance for breast and ovarian cancer

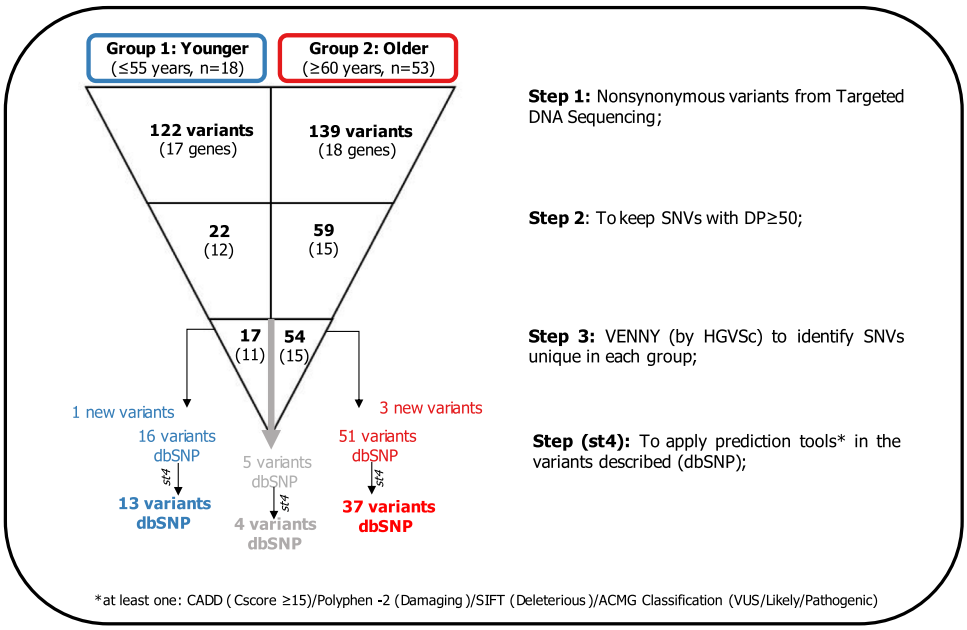

Fig. 3. Analysis workflow performed to describe the variants found in targeted DNA sequencing.

Table 1
Fourteen exclusive variants in the younger group after applied filters (Fig. 3).

Gene	dbSNP	HGVS coding	Consequence (HGVSp)	CADD (Cscore)	Reported as		
					PolyPhen-2	SIFT	ACMG classification
APC	rs113782655	c.78C > A	p.Ser26Arg	22,90	Unknown (0)	deleterious_low_confidence (0)	Benign
ATR	novel	c.2898C > A	p.His966Gln	22,30	Benign (0.083)	deleterious_low_confidence (0.03)	—
ATR	rs28897763	c.268C > T	p.His90Tyr	21,20	Benign (0.025)	tolerated_low_confidence (0.18)	Benign
ATR	rs763130593	c.5303A > G	p.Asp1768Gly	23,40	Benign (0.033)	Deleterious (0.03)	Likely benign
BRCA1	rs1396926041	c.3677T > C	p.Phe1226Ser	28,70	Benign (0.087)	Deleterious (0)	VUS
BRCA1	rs80356923	c.3640G > T	p.Glu1214Ter	37,00	—	—	Pathogenic
GFI1B	rs140090505	c.415G > T	p.Gly139Cys	24,90	probably_damaging (0.933)	Deleterious (0.02)	Likely benign
KLK3	rs61750343	c.373C > T	p.Arg125Cys	19,52	probably_damaging (0.91)	Deleterious (0.01)	Likely benign
KMT2C	rs61730547	c.7958T > C	p.Leu2653Pro	16,33	Benign (0)	—	Benign
KMT2D	rs146044282	c.10256A > G	p.Asp3419Gly	27,60	—	—	Benign
KMT2D	rs201507971	c.7705G > A	p.Gly2569Ser	15,06	—	—	Benign
KMT2D	rs777215324	c.10942C > T	p.Pro3648Ser	19,74	—	—	Benign
MLH1	rs771044689	c.1514G > A	p.Ser505Asn	25,70	Benign (0.404)	deleterious(0)	VUS
MSH6	rs34625968	c.3911G > A	p.Arg1304Lys	23,30	—	tolerated(0.21)	Benign

and Hereditary Cancer-Predisposing Syndrome. Similarly, another variant, rs1396926041, located in the *BRCA1* gene and classified as a VUS, showed clinical significance for Hereditary Breast Ovarian Cancer Syndrome. The *MLH1* variant rs771044689, also classified as VUS, showed clinical significance for hereditary cancer-predisposing syndrome, as reported in the ClinVar Database. Following the same stringent filtering process (Fig. 3) the older group of 50 cases yielded 37 exclusive variants. Of the 51 exclusive variants to this group, 37 (72%) were predicted as damaging/pathogenic according to at least one of these in silico prediction tools (Table 2).

3.5. Novel variant analysis in different age groups

This study revealed the presence of 4 novel variants. Of these, 1 is uniquely identified within the young group (ATR:c.2898C > A*),* while 3 are exclusively present in the older group *(*ATR:c.6265C > T, KMT2C:c.6782T > C and PTEN:c.10A>G*).* The c.6265C > T variant presents pathogenicity according to prediction tools and ACMG classification. In addition, the c.10A > G classified as VUS, was reported in association with hereditary cancer syndrome within the ClinVar Database.

3.6. Germline variants in DNA-repair genes (DRGs)

A total of 39 germline variants were found in 8 DRGs, in 35 of the 71 patients (49%). Of these, 10 were in the young group and 25 in the older group. Although the older group had a higher frequency of variants in DRGs compared to the young group (64% vs. 25%), our study does not provide evidence of a conclusive link between age stratification and the presence or absence of germline variants within DRGs (Chi-square Test, P-value = 0.582). The most frequently mutated DRG in the total sample was *BRCA2 (*23%), however, we did not find any variant with DP ≥ 50 in *BRCA2* in the younger group, which could be attributed to the relatively smaller size of the younger sample pool. It is possible that this discrepancy arises from the limited number of samples in the younger group. Intriguingly, the PARP1 gene exhibited variants exclusively within the young group, while the PALB2 gene exclusively displayed variants within the older group.

3.7. Survival analysis on PCa large cohort

Aiming to investigate the impact of the 2 the most mutated genes in our cohort on survival, we analyzed mutation data from 2 large cohorts of the ICGC prostate cancer project (PRAD-US + PRAD-UK, $n = 613$, available at: https://dcc.icgc.org/) for *BRCA2* and *KMT2C.* Disease-free survival (DFS) analysis showed a trend towards poorer disease-free survival in cases where mutations in *BRCA2* and *KMT2C* were detected in tumoral tissue (Supplementary Fig. 2).

Table 2
Forty exclusive variants in the older group after applied filters (Fig. 3).

Gene	dbSNP	HGVS coding	Consequence (HGVSp)	CADD (Cscore)	Reported as		
					PolyPhen-2	SIFT	ACMG classification
APC	rs139387758	c.4420G > A	p.Ala1474Thr	20,40	Benign (0)	tolerated_low_confidence (0.07)	Benign
APC	rs143638171	c.3386T > C	p.Leu1129Ser	23,70	Benign (0.163)	deleterious_low_confidence (0.01)	Benign
APC	rs147394539	c.2847G > T	p.Met949Ile	17,15	Benign (0)	tolerated_low_confidence (0.06)	Benign
APC	rs200587641	c.6857C > T	p.Ala2286Val	17,35	Benign (0.006)	tolerated_low_confidence (0.05)	Benign
APC	rs587780553	c.8038C > A	p.Pro2680Thr	23,30	probably_damaging (0.999)	deleterious_low_confidence (0.02)	VUS
APC	rs72541816	c.7862C > G	p.Ser2621Cys	23,10	Benign (0.245)	tolerated_low_confidence (0.06)	Benign
ATR	novel	c.6265C > T	p.Arg2089Ter	39,00	—	—	—
ATR	rs28910273	c.6394T>G	p.Tyr2132Asp	20,90	Benign (0.006)	Deleterious (0.03)	Benign
ATR	rs769648140	c.2437A > G	p.Met813Val	18,54	Benign (0.038)	Tolerated (0.14)	Likely benign
BRCA1	rs56214134	c.3600G > C	p.Gln1200His	17,84	Benign (0.185)	Deleterious (0.03)	Likely benign
BRCA2	rs11571833	c.9976A>T	p.Lys3326Ter	35,00	—	—	Benign
BRCA2	rs28897701	c.223G > C	p.Ala75Pro	24,40	possibly_damaging (0.574)	Deleterious (0)	Benign
BRCA2	rs4987048	c.5704G > A	p.Asp1902Asn	9,49	possibly_damaging (0.497)	Tolerated (0.12)	Benign
BRCA2	rs56087561	c.5070A > C	p.Lys1690Asn	23,20	possibly_damaging (0.607)	Deleterious (0)	Likely benign
BRCA2	rs59004709	c.8905G > A	p.Val2969Met	18,32	possibly_damaging (0.534)	Deleterious (0.03)	Benign
CDK12	rs200075664	c.1139G > A	p.Arg380His	28,60	Unknown (0)	deleterious_low_confidence (0)	Likely benign
GFI1B	rs114955344	c.100G > A	p.Val34Met	23,60	Benign (0.188)	Tolerated (0.14)	Benign
GFI1B	rs115534814	c.242G > T	p.Gly81Val	17,31	Benign (0.006)	Tolerated (0.25)	Benign
KLK3	rs61729813	c.629C > G	p.Ser210Trp	10,26	possibly_damaging (0.745)	Tolerated (0.1)	Benign
KMT2C	novel	c.6782T > C	p.Leu2261Ser	23,20	probably_damaging (0.994)	—	—
KMT2C	rs138119145	c.3955G > C	p.Asp1319His	25,80	possibly_damaging (0.884)	—	Benign
KMT2C	rs138845109	c.8502A > T	p.Glu2834Asp	9,14	probably_damaging (0.979)	—	Benign
KMT2C	rs140719911	c.6275A > T	p.Asp2092Val	23,20	probably_damaging (0.998)	—	Benign
KMT2C	rs547763902	c.10732C > A	p.His3578Asn	16,31	Benign (0.09)	—	Likely benign
KMT2C	rs567984906	c.10234C > T	p.Arg3412Trp	25,30	probably_damaging (0.995)	—	VUS
KMT2C	rs61730540	c.6653A > C	p.Tyr2218Ser	24,50	probably_damaging (0.996)	—	Benign
KMT2D	rs181733689	c.13045C > G	p.Pro4349Ala	17,08	—	—	Benign
KMT2D	rs563981206	c.6437C > T	p.Pro2146Leu	24,80	—	—	Benign
KMT2D	rs777559590	c.12940C > T	p.Pro4314Ser	23,40	—	—	Likely benign
KMT2D	rs780460242	c.7521T > A	p.His2507Gln	16,00	—	—	Likely benign
MLH1	rs2308317	c.637G > A	p.Val213Met	23,70	Benign (0.105)	Deleterious (0.03)	Benign
MSH6	rs267608075	c.3160A > T	p.Ile1054Phe	20,60	—	Tolerated (0.13)	Likely benign
NBN	rs34767364	c.643C > T	p.Arg215Trp	25,90	—	Deleterious (0)	Benign
NBN	rs61753720	c.283G > A	p.Asp95Asn	23,70	—	deleterious(0.01)	VUS
NBN	rs61754796	c.628G > T	p.Val210Phe	15,27	—	Deleterious (0.02)	Likely benign
NBN	rs769420	c.797C > T	p.Pro266Leu	24,50	—	Deleterious (0)	Benign
PALB2	rs138789658	c.53A > G	p.Lys18Arg	25,10	probably_damaging (0.986)	Deleterious (0)	Benign
PALB2	rs780415750	c.2596G > T	p.Gly866Cys	22,80	possibly_damaging (0.857)	Deleterious (0.04)	VUS
PTEN	novel	c.10A > G	p.Ile4Val	19,62	Benign (0)	Tolerated (0.53)	—
PTEN	rs143335584	c.882T > G	p.Ser294Arg	23,90	Benign (0.178)	Tolerated (0.05)	Likely benign

4. Discussion

Racial and ethnic differences directly impact cancer incidence and mortality worldwide. Investigating patient and tumor features that underlie well−established prostate cancer racial disparities holds promise to identify risk factors not only for carcinogenesis but also for the observed therapeutic response differences.

This study is distinct due to its focus on younger Brazilian PCa patients, representing a crucial gap in the existing research landscape concerning germline mutations. Given Brazil's diverse population, our findings are of particular importance.

The most mutated genes in our cohort were *BRCA2* and *KMT2C*. *BRCA2* mutations are well-established genetic susceptibility factors in PCa [11]. Our study is in accordance with previous ones, as *BRCA2* is the most frequently subjected gene in the blood cells of PCa patients [11,12]. In our cohort, among eleven variants located in *BRCA2*, 5 (rs56087561, rs28897701, rs4987048, rs59004709, and rs11571833) are classified as pathogenic/damaging in at least one of the silico prediction tools. The rare truncating variant (rs11571833) known as K3326X, found here, has been previously observed in a Brazilian individual with breast cancer diagnosed at 35 years old [13] and linked to an elevated risk of various solid tumors such as breast [14], pancreas [15], and bladder [16]. While its association with PCa, remains less established, our findings suggest a potential increase in PCa risk among Brazilian men harboring this variant.

A significant finding of our study was the high frequency of *KMT2C* mutations. This is particularly interesting because, to our knowledge, the germline mutations found in our study have not been previously reported. Only 1 study with Chinese PCa patients identified germline mutations in the *KMT2C* gene. However, they found 2 different mutations (rs200662726 and rs752118948) [17]. Conversely, somatic alterations in PCa tumoral tissue have already been described [18]. However, it is important to acknowledge that the germline mutations we detected in *KMT2C* may potentially represent clonal hematopoiesis variants, as our analysis was conducted on DNA extracted from blood cells. *KMT2C* has recently been recognized as being susceptible to mutations in clonal hematopoiesis [19].

The *KMT2C* gene encodes a histone-lysine N-methyltransferase that increases chromatin accessibility for transcriptional machinery, facilitating the expression of genes [20,21]. Although *KMT2C* has been described as the most mutated epigenetic regulator and driver in PCa tumoral tissue [22], germline mutations found in this study have not been previously described. Among the known variants located in the *KMT2C* gene, 7 presented as pathogenic/damaging in at least one of the bioinformatics prediction tools used (rs61730547, rs140719911, rs138119145, rs61730540, rs138845109, rs547763902, and rs567984906). Notably, rs61730547 and rs138845109 variants have been reported previously in a study that aimed to characterize the germline variation in cancer-susceptibility genes [23]. Moreover, our investigation identified a novel variant in *KMT2C* (p. Leu2261Ser), predicted as pathogenic by in silico tools, although this finding needs further validation. Germline variants within the *KMT2C* gene have previously been associated with cancer risk in families with suspected hereditary cancer [24].

The *BRCA2* is well-established as a tumor suppressor gene in PCa. Carriers of germline mutations in BRCA2 face an elevated risk of developing PCa and experiencing unfavorable outcomes, compared to noncarriers [25]. Our survival analysis was consistent with others that have associated *BRCA2* mutations with poor survival outcomes [26]. In addition, studies involving breast and colorectal cancer suggest that *KMT2C* also may act as a tumor suppressor [27,24]. Similarly, Limberg and colleagues [18] analyzed PCa data from ICGG (International Cancer Genome Consortium) and found that truncated *KMT2C* mutations are correlated with reduced disease-free survival, which is consistent with our findings. The same correlation has already been reported for other types of solid tumors [28,29]. The characterization of KMT2A, KMT2B, KMT2C, and KMT2D pathogenic variation described by Larson and colleagues suggests that these variants are more common than previous findings [30]. Taken together, these data indicate that the *KMT2C* gene may assume a role as crucial as *BRCA2* in tumoral suppression in PCa patients.

No germline variants were found in *TP53, FOXA1,* and *SPOP* genes in our study. However, these genes were previously identified as recurrently mutated in PCa tumor tissue [11,31]. This observation further supports the idea that these genes may not play a role in genetic susceptibility, despite their crucial role in the carcinogenesis process, evidenced by their somatic mutations in tumor tissue. Additionally, no germline variants were found in the *HOXB13* gene. The rare germline variant G84E described for the first time by Ewing and colleagues [32] is widely known to confer genetic susceptibility for PCa in men with European ancestry. Even though G84E is associated with early PCa ($\leq$ 55 years) [32−33], and our cohort includes patients from regions in Brazil that are marked by a strong European ancestry, our study does not provide evidence of the presence of this variant in Brazilian men. In line with this, so far there are no other Brazilian studies reporting this variant. Additionally, the most prevalent variants were the rs35001569 and rs63750449 in *MLH1*, both identified in 3 patients. The *MLH1* gene encodes one of the complex members of the mismatch repair pathway [34], and germline mutations in this gene are associated with an increased risk to develop cancer [35]. Although the conflicting interpretations of pathogenicity for rs35001569 in the ClinVar database, it was predicted to be damaging in our analysis, and presents allele frequency < 0.01 in 1000Gp3, gnomAD, ExAC, and ABRaOM datasets. Furthermore, this variant

was also found in a Brazilian study analyzing germline *MLH1* variants in patients with colorectal cancer [36]. Joy and colleagues (2014) [37] identified rs35001569 in patients with colon cancer as the most deleterious among the mutations in *MLH1*. Last, this variant was presented as a driver mutation in breast cancer patients from Southwest Colombia [38] and identified as a potential susceptibility variant in familial PCa cases [39]. Collectively, these data imply that rs35001569 may contribute to genetic susceptibility in PCa, particularly in patients diagnosed at ≤ 55 years old.

The major limitation of this study is the small number of younger participants, although this is a very common frailty in PCa studies. Second, the disease-free survival analysis was not performed with the same patients as in the DNA sequencing, due to the limited time of follow-up.

5. Conclusions

This study successfully identified exclusive and deleterious germline variants in early-onset and late-onset PCa patients, contributing to a deeper understanding of this cancer in Brazilian patients. These results provide a resource for further investigations and validations in larger Brazilian cohorts. To our knowledge, this study is the first to report functionally deleterious germline variants in the *KMT2C* gene in PCa patients.

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Ethics approval and consent to participate

The study was evaluated and approved by the Ribeirao Preto Medical School, University of São Paulo, and Erasto Gaertner Hospital ethics committee. All blood samples were collected at diagnosis with patients' informed consent. This study complies with the Declaration of Helsinki and was performed according to ethics committee approval.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

CRediT authorship contribution statement

Jeremy A. Squire: Writing – review & editing. **Kelly Gomes Duarte:** Methodology. **Cláudia Tarcila Gomes Sares:** Methodology. **Natalia Alonso Moreda:** Formal analysis. **Jonatas Luiz Pereira:** Data curation. **Israel Tojal da Silva:** Formal analysis. **Alexandre Defelicibus:** Formal analysis. **Mateus Nóbrega Aoki:** Writing – review & editing. **Javier De Las Rivas:** Writing – review & editing. **Rodolfo Borges dos Reis:** Data curation. **Dalila Lucíola Zanette:** Conceptualization, Data curation, Funding acquisition, Project administration, Supervision, Writing – review & editing.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.urolonc.2024.01.015.

References

[1] Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021;71(3):209–49.
[2] Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2021. Ca Cancer J Clin 2021;71(1):7–33.
[3] Instituto Nacional De Câncer. Programa Saber Saúde. Rio de Janeiro: INCA, 2023. Disponível em: <Available at: https://www.inca.gov.br/programa-nacional-de-controle-do-tabagismo/programa-saber-saude>.
[4] Rawla P. Epidemiology of prostate cancer. World J Oncol 2019;10(2):63.
[5] Mucci LA, Hjelmborg JB, Harris JR, Czene K, Havelick DJ, Scheike T, Kaprio J. Familial risk and heritability of cancer among twins in Nordic countries. JAMA 2016;315(1):68–76.
[6] Dias A, Kote-Jarai Z, Mikropoulos C, Eeles R. Prostate cancer germline variations and implications for screening and treatment. Cold Spring Harb Perspect Med 2018;8(9).
[7] SALINAS Claudia A, et al. Prostate cancer in young men: an important clinical entity. Nat Rev Urol 2014;11(6):317–23.
[8] KIRCHER Martin, et al. A general framework for estimating the relative pathogenicity of human genetic variants. Nat Genet 2014;46(3):310–5.
[9] Adzhubei I, Schmidt S, Peshkin L, Ramensky V, Gerasimova A, Bork P, Sunyaev SR. PolyPhen-2: prediction of functional effects of human nsSNPs. Nat Methods 2010;7(4).
[10] Ng PC, Henikoff S. SIFT: Predicting amino acid changes that affect protein function. Nucleic Acids Res 2003;31(13):3812–4.
[11] Abida W, Armenia J, Gopalan A, Brennan R, Walsh M, Barron D, Scher HI. Prospective genomic profiling of prostate cancer across

disease states reveals germline and somatic alterations that may affect clinical decision making. JCO Precis Oncol 2017;1:1–16.
[12] Nicolosi P, Ledet E, Yang S, Michalski S, Freschi B, O'Leary E, Sartor O. Prevalence of germline variants in prostate cancer and implications for current genetic testing guidelines. JAMA Oncol 2019;5(4):523–8.
[13] da Costa e Silva Carvalho S, Cury NM, Brotto DB, De Araujo LF, Rosa RCA, Texeira LA, Silva WA Jr. Germline variants in DNA repair genes associated with hereditary breast and ovarian cancer syndrome: Analysis of a 21 gene panel in the Brazilian population. BMC Med Genet 2020;13:1–24.
[14] Thompson ER, Gorringe KL, Rowley SM, et al. Reevaluation of the BRCA2 truncating allele c. 9976A>T (p. Lys3326Ter) in a familial breast cancer context. Sci Rep 2015;5(1):14800.
[15] Obazee O, Archibugi L, Andriulli A, et al. Germline BRCA2 K3326X and CHEK2 I157T mutations increase risk for sporadic pancreatic ductal adenocarcinoma. Int J Cancer 2019;145(3):686–93.
[16] Ge Y, Wang Y, Shao W, et al. Rare variants in BRCA2 and CHEK2 are associated with the risk of urinary tract cancers. Sci Rep 2016;6 (1):33542.
[17] Liang Y, Chiu PKF, Zhu Y, Wong CYP, Xiong Q, Wang L, Ng CF. Whole-exome sequencing reveals a comprehensive germline mutation landscape and identifies twelve novel predisposition genes in Chinese prostate cancer patients. PLos Genet 2022;18(9):e1010373.
[18] Limberger T, Schlederer M, Trachtová K, Garces de los Fayos Alonso I, Yang J, Högler S, Kenner L. KMT2C methyltransferase domain regulated INK4A expression suppresses prostate cancer metastasis. Mol Cancer 2022;21(1):89.
[19] Pich O, Reyes-Salazar I, Gonzalez-Perez A, Lopez-Bigas N. Discovering the drivers of clonal hematopoiesis. Nat Commun 2022;13(1):4267.
[20] Cho SJ, Yoon C, Lee JH, Chang KK, Lin JX, Kim YH, Yoon SS. KMT2C mutations in diffuse-type gastric adenocarcinoma promote epithelial-to-mesenchymal transition. Clin Cancer Res 2018;24 (24):6556–69.
[21] Lavery WJ, Barski A, Wiley S, Schorry EK, Lindsley AW. KMT2C/D COMPASS complex-associated diseases [K CD COM-ADs]: an emerging class of congenital regulopathies. Clin Epigenet 2020;12:1–20.
[22] Armenia J, Wankowicz SA, Liu D, Gao J, Kundra R, Reznik E, Van Allen EM. The long tail of oncogenic drivers in prostate cancer. Nat Genet 2018;50(5):645–51.
[23] Bodian DL, McCutcheon JN, Kothiyal P, Huddleston KC, Iyer RK, Vockley JG, Niederhuber JE. Germline variation in cancer-susceptibility genes in a healthy, ancestrally diverse cohort: implications for individual genome sequencing. PLoS One 2014;9(4):e94554.
[24] Larsson C, Cordeddu L, Siggens L, Pandzic T, Kundu S, He L, Sjöblom T. Restoration of KMT2C/MLL3 in human colorectal cancer cells reinforces genome-wide H3K4me1 profiles and influences cell growth and gene expression. Clin Epigenet 2020;12(1):1–12.
[25] Oh M, Alkhushaym N, Fallatah S, Althagafi A, Aljadeed R, Alsowaida Y, Abraham I. The association of BRCA1 and BRCA2 mutations with prostate cancer risk, frequency, and mortality: A meta-analysis. Prostate 2019;79(8):880–95.
[26] Castro E, Goh C, Olmos D, Saunders E, Leongamornlert D, Tymrakiewicz M, Eeles R. Germline BRCA mutations are associated with higher risk of nodal involvement, distant metastasis, and poor survival outcomes in prostate cancer. J Clin Oncol 2013;31(14):1748.
[27] Gala K, Li Q, Sinha A, Razavi P, Dorso M, Sanchez-Vega F, Chandarlapaty S. KMT2C mediates the estrogen dependence of breast cancer through regulation of ER enhancer function. Oncogene 2018;37 (34):4692–710.
[28] Chen X, Zhang G, Chen B, Wang Y, Guo L, Cao L, Liao N. Association between histone lysine methyltransferase KMT2C mutation and clinicopathological factors in breast cancer. Biomed Pharmacother 2019;116:108997.
[29] Liu X, Qiu R, Xu M, Meng M, Zhao S, Ji J, Yang Y. KMT2C is a potential biomarker of prognosis and chemotherapy sensitivity in breast cancer. Breast Cancer Res Treat 2021;189:347–61.
[30] Larson JK, Hunter-Schlichting DN, Crowgey EL, Mills LJ, Druley TE, Marcotte EL. KMT2A-D pathogenicity, prevalence, and variation according to a population database. Cancer Med 2023;12 (6):7234–45.
[31] Barbieri CE, Baca SC, Lawrence MS, Demichelis F, Blattner M, Theurillat JP, Garraway LA. Exome sequencing identifies recurrent SPOP, FOXA1 and MED12 mutations in prostate cancer. Nat Genet 2012;44(6):685–9.
[32] Ewing CM, Ray AM, Lange EM, Zuhlke KA, Robbins CM, Tembe WD, Cooney KA. Germline mutations in HOXB13 and prostate-cancer risk. N Engl J Med 2012;366(2):141–9.
[33] Xu J, Lange EM, Lu L, et al. International Consortium for Prostate Cancer Genetics. HOXB13 is a susceptibility gene for prostate cancer: results from the International Consortium for Prostate Cancer Genetics (ICPCG). Hum Genet 2013;132:5–14.
[34] Lipkin SM, Wang V, Jacoby R, Banerjee-Basu S, Baxevanis AD, Lynch HT, Collins FS. MLH3: a DNA mismatch repair gene associated with mammalian microsatellite instability. Nat Genet 2000;24 (1):27–35.
[35] Sharma M, Yang Z, Miyamoto H. Loss of DNA mismatch repair proteins in prostate cancer. Medicine 2020;99(19).
[36] Schneider NB, Pastor T, Paula AE D, et al. Germline MLH 1, MSH 2 and MSH 6 variants in Brazilian patients with colorectal cancer and clinical features suggestive of Lynch Syndrome. Cancer Med 2018;7 (5):2078–88.
[37] JJoy A, Jubil C, Syama P, Menon R. MLH1 gene: An in silico analysis. J Comput Biol Bioinform Res 2013;5(1):1–5.
[38] Cortes-Urrea C, Bueno-Gutiérrez F, Solarte M, et al. Exomes of ductal luminal breast cancer patients from southwest colombia: gene mutational profile and related expression alterations. Biomolecules 2020;10(5):698.
[39] Johnson AM, Zuhlke KA, Plotts C, McDonnell SK, Middha S, Riska SM, Cooney KA. Mutational landscape of candidate genes in familial prostate cancer. Prostate 2014;74(14):1371–8.