Increased Risk of Hereditary Prostate Cancer in Italian Families with Hereditary Breast and Ovarian Cancer Syndrome Harboring Mutations in \textit{BRCA} and in Other Susceptibility Genes

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Abstract: Hereditary prostate cancer (HPCa) has the highest heritability of any cancer in men. Interestingly, it occurs in several hereditary syndromes, including breast and ovarian cancer (HBOC) and Lynch syndrome (LS). Several gene mutations related to these syndromes have been identified as biomarkers in HPCa. The goal of this study was to screen for germline mutations in susceptibility genes by using a multigene panel, and to subsequently correlate the results with clinical and laboratory parameters. This was undertaken in 180 HBOC families, which included 217 males with prostate cancer (PCa). Mutational analysis was further extended to 104 family members of mutated patients. Screening of HBOC families revealed that 30.5% harbored germline mutations in susceptibility genes, with 21.6% harboring pathogenic variants (PVs) and 8.9% having variants of uncertain significance (VUS). We found PVs at similar frequency in $\textit{BRCA1}$ and $\textit{BRCA2}$ genes (8.8% and 9.4%, respectively), while 0.56% of PVs were present in well-established susceptibility genes $\textit{PALB2}$, $\textit{TP53}$ and $\textit{RAD51C}$. Moreover, 0.56% of monoallelic PVs were present in $\textit{MUTYH}$, a gene whose function in tumorigenesis in the context of PCa is still unclear. Finally, we reported double heterozygosity (DH) in $\textit{BRCA1}/2$ genes in a single family, and found double mutation (DM) present in $\textit{BRCA2}$ in a separate family. There was no significant difference between the mean age of onset of PCa in HBOC families with or without germline mutations in susceptibility genes, while the mean survival was highest in mutated patients compared to wild type. Furthermore, PCa is the second most recurrent cancer in our cohort, resulting in 18% of cases in both mutated and non-mutated families. Our investigation shows that PVs were located mostly in the 3' of $\textit{BRCA1}$ and $\textit{BRCA2}$ genes, and in $\textit{BRCA2}$, most PVs fell in exon 11, suggesting a mutation cluster region relating to risk of HPCa. A total of 65 family members inherited the proband's mutation; of these, 24 developed cancer, with 41 remaining unaffected.

Keywords: hereditary prostate cancer; hereditary breast and ovarian cancer syndrome; $\textit{BRCA}$ genes; susceptibility genes

1. Introduction

Prostate cancer (PCa) is a heterogeneous disease, representing one of the major causes of malignancy-related deaths worldwide, with about 1,600,000 cases and 366,000 deaths annually [1]. Men with first-degree male relatives affected by PCa have at least a two-fold risk of developing PCa compared to men without a family history of PCa [2]. Approximately 5–15% of PCa cases are attributable to hereditary factors [3]. Hereditary prostate cancer (HPCa) has the highest heritability of any cancer in men, with tumorigenesis occurring due to
the presence of germline mutations transmitted through autosomal dominant inheritance [4]. In comparison to sporadic cases, HPCa shows an early age of onset, an aggressive disease progression and locally advanced stage, with a higher risk of recurrence after surgery.

HPCa arises in several hereditary syndromes, including hereditary breast and ovarian cancer (HBOC) and Lynch syndrome (LS) [5]. Several genes related to these syndromes, such as DNA damage repair (DDR) genes BRCA1, BRCA2, CHEK2, ATM and PALB2, and DNA mismatch repair (MMR) genes MLH1, MSH2, MSH6 and PMS2, were identified as biomarkers in HPCa. However, mutations in HOXB13, BRP1, NBS1, RAD51C, RAD51D and TP53 genes have been found to confer an increased risk of HPCa [6–9].

The mutation status of genes involved in HPCa could have potential clinical applications, particularly for the stratification of patients into specific treatment and prognostic groups. Patients who harbor mutations in DDR genes, for example, would likely be more sensitive to a treatment regime that includes PARP inhibitors, whereas the mutational status of MMR genes in metastatic patients could predict their response to immunotherapy [10].

Current guidelines do not adequately inform at what disease stage genetic testing should be performed to identify germline mutations in patients who could potentially benefit from alternative treatment. Moreover, the identification of germline mutations in a patient with HPCa could facilitate the genetic testing of family members at risk of HPCa or other associated cancers.

The goal of the study was to identify germline mutations insusceptibility genes using a multigene panel in HBOC Italian families with at least one case of PCa by correlating clinical and laboratory parameters. Mutational analysis was extended to family members of the mutated patients.

2. Materials and Methods

2.1. Patients

This study was carried out in accordance with the World Medical Association Helsinki Declaration (1964). Informed consent was obtained from all subjects, and the study was approved and conducted according to the ethical guidelines of the University of Campania “Luigi Vanvitelli” (n. 469-23 July 2019). The study was conducted at the U.O.C. Clinical and Molecular Pathology, A.O.U. University of Campania “Luigi Vanvitelli”.

We enrolled 180 HBOC families that included 217 males with PCa. The probands were affected with prostate, breast, ovarian, colorectal, pancreatic, endometrial, bladder, gastric cancer, or melanoma (Table 1), with an age range of 28–86 years. All selected patients received genetic counselling. Case and family history were collected, and a pedigree was generated for each family. The patients were selected according to the criteria for HBOC syndrome [11], in which mutations occur in high-penetrance BRCA1/2 genes, followed by PALB2, and mutations in genes that confer moderate penetrance risk, such as ATM, CHEK2, RAD51C, RAD50, BRIP1, PTEN, NBN, MRE11A, BARD1, STK11, CDH1, MUTYH and TP53. Peripheral blood samples were collected in two test EDTA tubes from all patients. Mutational analysis was extended to 104 family members of 34 mutated patients.

Table 1. Number and type of HBOC probands.

| Syndrome                                      | No. of Families | No. and Tumor Type of Probands                                                                 | No. of PCa Cases |
|-----------------------------------------------|-----------------|-------------------------------------------------------------------------------------------------|------------------|
| Hereditary breast and ovarian cancer syndrome (HBOC) | 180             | 154 breast cancers<br>3 ovarian cancers<br>3 colorectal cancers<br>3 melanomas<br>2 prostate cancers<br>2 prostate and colorectal cancers<br>2 prostate, colorectal and bladder cancers<br>2 endometrial cancers<br>2 ovarian and colorectal cancers<br>1 pancreatic cancer<br>1 gastric cancer | 217               |
2.2. Mutational Analysis

Genomic DNA from blood samples was extracted using the Wizard Genomic DNA purification kit (Promega Corporation, Madison, WI, USA) according to the manufacturer’s instructions. For mutational analysis, we used the TruSight Sequencing Cancer Panel on a MiSeq platform (Illumina, HCS, Sophia Genetics, Switzerland) that analyzes 16 genes, **BRCA1** (NM_007295), **BRCA2** (NM_000059), **ATM** (NM_000051.4), **CHEK2** (NM_007194), **PALB2** (NM_024675), **RAD51C** (NM_058216), **RAD50** (NM_002878), **BRP1** (NM_001003694.2), **PTEN** (NM_000314.8), **NBN** (NM_002485), **MRE11A** (NM_005591.4), **CDH1** (NM_004360), **MUTYH** (NM_001128425.1) and **TP53** (NM_000546). The presence of point mutation was confirmed on the other blood sample by Sanger sequencing, as previously described [12]. Molecular analysis in family members of mutated probands was performed by Sanger sequencing, as previously described [13,14]. The results were analyzed using Mutation Surveyor® software, version 3.24 (Softgenetics, State College, PA, USA).

2.3. Genetic Variant Classification

ClinVar and LOVD databases were used for the identification and classification of genetic variants. Genetic variants found were categorized according to criteria developed by International Agency for Research on Cancer recommendations [15] and categorized into five classes: benign (class I), likely benign (class II), variant of uncertain significance (VUS, class III), likely pathogenic (class IV) and pathogenic variants (PVs, class V).

3. Results

Mutational screening conducted in 180 HBOC families revealed 55 (30.5%) germline mutations in 51 patients. These mutations included 39/180 (21.6%) PV and 16/180 (8.9%) VUS (Figure 1). Moreover, 20/39 (51.3%) of the observed PVs were frameshift, 6/39 (15.4%) intronic variants, 6/39 (15.4%) nonsense, 6/39 (15.4%) missense and 1/39 (2.5%) copy number variation (CNV).

![Figure 1. Pathogenic variants and VUS found in HBOC families.](image-url)

3.1. Mutations in DNA Damage Repair Genes

From the families carrying PVs, 16/180 (8.8%) harbored a PV in **BRCA1**, 17/180 (9.4%) in **BRCA2**, 1/180 (0.56%) in **PALB2**, 1/180 (0.56%) in **TP53** and 1/180 (0.56%) in **RAD51C**. We also observed double heterozygosity (DH) in **BRCA1/2** genes (0.56%) in a single family, and found double mutations (DM) present in **BRCA2** (0.56%) in a separate family (Figure 1).

The **BRCA1** mutation c.5123C>A(p.Ala1708Glu) was found in three separate families and c.5266dupC (p.Gln1756Profs) in four families; the **BRCA2** mutation c.4133_4136del (p.Thr1378fs) was observed in two families and c.6468_6469delTC (p.Gln2157Ilefs) in three families.
3.2. Mutations in Base Excision Repair Gene

In the families with observed PVs, 1/180 (0.56%) carried a monoallelic PV in the MUTYH gene (Figure 1). This specific mutation consists of a frameshift deletion in exon 14 of the gene (between nucleotides 1437 and 1439), resulting in the introduction of a premature stop codon at amino acid position 480. It was identified in an 85-year-old woman diagnosed with breast cancer at the age of 65. Figure 2 reports the pedigree analysis of the proband; her brother, affected with prostate cancer, inherited the mutation. In addition, the mutational analysis was extended to the daughter, who was diagnosed with endometrial cancer at 55 years of age, and to the unaffected son of 58 years, revealing that the mutation was also present in both the daughter and son.

![Pedigree](Image)

**Figure 2.** Pedigree of the proband carrying the monoallelic MUTYH mutation c.1437_1439del GGA (p.Glu480del). The ages at diagnosis are indicated in brackets.

3.3. Genotype–Phenotype Correlation

We observed a mean age of PCa onset of 67.4 years in mutated patients and 68.3 years in non-mutated patients. Of the 217 cases in the cohort, 66 patients died. The mean survival was 8 years and was higher in mutated patients (77.5 years) than in non-mutated patients (74.1 years). Patients who were carriers of VUS were included in the non-mutated patient group (Figure 3).

![Box plot](Image)

**Figure 3.** Onset age of 217 PCa patients. HBOC-mutated families included 49 individuals, and non-mutated families 168 individuals. Age of death of 66 PCa patients is also illustrated in this plot.
The number and percentage of cancer types that occur in mutated and non-mutated HBOC families are shown in Figure 4. PCa is the second most recurrent cancer, resulting in 18% of cases in both mutated and non-mutated families.

Figure 4. (A) Cancer types that occur in HBOC-mutated families. (B) Cancer types that occur in HBOC non-mutated families. BC: breast cancer; PCa: prostate cancer; OC: ovarian cancer; LC: lung cancer; GC: gastric cancer; EC: endometrial cancer; LAC: laryngeal cancer; CC: colon cancer; PDAC: pancreatic ductal adenocarcinoma; BRC: brain cancer; BLC: bladder cancer; LEU: leukemia; MEL: melanoma; TYC: thyroid cancer; BNC: bone cancer; KC: kidney cancer; TC: testicular cancer; Sr: sarcoma; ESC: esophageal cancer.

Figure 5 illustrates the location of PVs identified in our cohort in both the \textit{BRCA1} and \textit{BRCA2} genes. In both genes, the mutations are located throughout the length of gene, mostly in 3\textsuperscript{'}. In \textit{BRCA2}, most of them fall in exon 11.

Mutational analysis was extended to 104 family members of 30 proband patients harboring mutations in \textit{BRCA1} and/or \textit{BRCA2} genes and four proband patients who were carriers of mutations in \textit{TP53}, \textit{PALB2}, \textit{RAD51C} or \textit{MUTYH} genes. This included both cancer-free family members and family members who were diagnosed with cancer. In the remaining eight families with \textit{BRCA} mutations, it was not possible to extend the mutational analysis to family members. The results of the genetic testing are summarized in Table 2. A total of 39 family members only displayed the wild-type gene, while 65 had inherited the proband’s mutation. Moreover, 24 of the 65 individuals who inherited the mutations of the proband developed cancer. The analysis was also conducted in families with DH and DM, showing that these pathogenic variants co-segregate.
Figure 4. (A) Cancer types that occur in HBOC-mutated families. (B) Cancer types that occur in HBOC non-mutated families. BC: breast cancer; PCa: prostate cancer; OC: ovarian cancer; LC: lung cancer; GC: gastric cancer; EC: endometrial cancer; LAC: laryngeal cancer; CC: colon cancer; PDAC: pancreatic ductal adenocarcinoma; BRC: brain cancer; BLC: bladder cancer; LEU: leukemia; MEL: melanoma; TYC: thyroid cancer; BNC: bone cancer; KC: kidney cancer; TC: testicular cancer; Sr: sarcoma; ESC: esophageal cancer.

Figure 5. Pathogenic variants found in HBOC families with PCa localized through BRCA1 and BRCA2 genes.

Table 2. Results of mutational analysis conducted in family members of 34 HBOC-mutated patients, with all pathogenic variants identified. The names of mutations are reported in bold.

| Family | Gene | Mutation | Exon | Family Members (Diagnosis) | Age | Mutational Analysis |
|--------|------|----------|------|---------------------------|-----|--------------------|
| 1      | BRCA1| c.117_118del (p.Cys39_Asp40delinsTer) | 3    | Proband (Ovarian Cancer) 65 | Mutated |
|        |      |          |      | Daughter (Unaffected) 41    | Wild Type |
| 2      | BRCA1| c.181T > G (p.Cys61Gly) | 5    | Proband (Breast Cancer) 30  | Mutated |
|        |      |          |      | Father (Prostate Cancer) 52 | Mutated |
|        |      |          |      | Sister (Unaffected) 31      | Wild Type |
|        |      |          |      | Sister (Unaffected) 27      | Wild Type |
|        |      |          |      | Sister (Unaffected) 21      | Wild Type |
| 3      | BRCA1| c.213-11T > G (IVS5-11T > G) | 6    | Proband (Ovarian Cancer) 55  | Mutated |
|        |      |          |      | Son (Unaffected) 28         | Wild Type |
|        |      |          |      | Sister (Unaffected) 24       | Mutated |
|        |      |          |      | Sister (Unaffected) 59       | Mutated |
| 4      | BRCA1| c.547 + 2T > A (IVS8 + 2T > A) | 8    | Proband (Breast Cancer) 52   | Mutated |
|        |      |          |      | Mother (Breast Cancer) 45†   |       |
|        |      |          |      | Aunt (Prostate Cancer) 60†   |       |
|        |      |          |      | Aunt (Breast Cancer) 48†      |       |
| 5      | BRCA1| c.798_799delTT (p.Ser267Lysfs) | 11   | Proband (Breast and Ovarian Cancer) | 56 | Mutated |
|        |      |          |      | Son (Unaffected) 28          | Mutated |
|        |      |          |      | Sister (Unaffected) 65       | Wild Type |
|        |      |          |      | Brother (Unaffected) 61       | Mutated |
| 6      | BRCA1| c.1796_1800del (p.Ser599*) | 11   | Proband (Ovarian Cancer) 58   | Mutated |
|        |      |          |      | Sister (Unaffected) 55       | Wild Type |
| 7      | BRCA1| c.3351dup (p.Gln1118fs) | 11   | Proband (Breast Cancer) 50   | Mutated |
|        |      |          |      | Cousin (Prostate Cancer) 53  | Mutated |
| 8      | BRCA1| c.3607 C > T (p.Arg1203Ter) | 11   | Proband (Breast Cancer) 40   | Mutated |
|        |      |          |      | Sister (Unaffected) 39       | Mutated |
|        |      |          |      | Niece (Unaffected) 18        | Wild Type |
| 9      | BRCA1| c.3756_3759delGTCT (p.Ser1253ArgfsTer10) | 11 | Proband (Ovarian Cancer) 59 | Mutated |
|        |      |          |      | Son (Unaffected) 36          | Wild Type |
|        |      |          |      | Daughter (Unaffected) 39      | Wild Type |
| 10     | BRCA1| c.5123C > A (p.Ala1708Glu) | 18   | Proband (Breast and Ovarian Cancer) | 52 | Mutated |
|        |      |          |      | Sister (Unaffected) 54       | Mutated |
|        |      |          |      | Brother (Unaffected) 51       | Wild Type |
Table 2. Cont.

| Family | Gene | Mutation | Exon | Family Members (Diagnosis) | Age  | Mutational Analysis |
|--------|------|----------|------|----------------------------|------|--------------------|
| 11     | BRCA1| c.5123C > A (p.Ala1708Glu) | 18   | Proband (Ovarian Cancer)   | 52   | Mutated            |
|        |      |          |      | Sister (Melanoma)          | 55   | Mutated            |
|        |      |          |      | Sister (Unaffected)        | 47   | Wild Type          |
|        |      |          |      | Cousin (Unaffected)        | 50   | Wild Type          |
| 12     | BRCA1| c.5123C > A (p.Ala1708Glu) | 18   | Proband (Colon Cancer)     | 54   | Mutated            |
|        |      |          |      | Sister (Unaffected)        | 55   | Mutated            |
|        |      |          |      | Sister (Unaffected)        | 59   | Mutated            |
|        |      |          |      | Cousin (Colon Cancer)      | 54   | Wild Type          |
| 13     | BRCA1| c.5266dupC (p.Gln1756Profs) | 20   | Proband (Breast and Ovarian Cancer) | 64   | Mutated            |
|        |      |          |      | Daughter (Unaffected)      | 36   | Wild Type          |
|        |      |          |      | Son (Unaffected)           | 38   | Mutated            |
|        |      |          |      | Brother (Unaffected)       | 66   | Mutated            |
|        |      |          |      | Niece (Breast Cancer)       | 32   | Mutated            |
|        |      |          |      | Niece (Prostate Cancer)     | 41   | Mutated            |
| 14     | BRCA1| c.5266dupC (p.Gln1756Profs) | 20   | Proband (Breast Cancer)     | 51   | Mutated            |
|        |      |          |      | Daughter (Unaffected)      | 28   | Mutated            |
| 15     | BRCA1| c.5266dupC (p.Gln1756Profs) | 20   | Proband (Ovarian Cancer)    | 43   | Mutated            |
|        |      |          |      | Sister (Unaffected)        | 27   | Wild Type          |
|        |      |          |      | Brother (Unaffected)       | 39   | Mutated            |
|        |      |          |      | Cousin (Breast and Ovarian Cancer) | 46   | Mutated            |
|        |      |          |      | Cousin (Unaffected)        | 57   | Wild Type          |
|        |      |          |      | Cousin (Unaffected)        | 54   | Wild Type          |
|        |      |          |      | Cousin (Prostate Cancer)    | 50   | Mutated            |
| 16     | BRCA1| c.547 + 2T > A (IVS8 + 2T > A) | 8    | Proband (Bilateral Breast Cancer) | 32   | Mutated            |
|        | BRCA2| c.2830A > T (p.Lys944Ter)     | 11   | Proband (Breast Cancer)     | 60   | Mutated            |
|        |      |          |      | Son (Unaffected)           | 56   | Mutated            |
|        |      |          |      | Daughter (Unaffected)      | 54   | Wild Type          |
|        |      |          |      | Daughter (Unaffected)      | 51   | Wild Type          |
| 17     | BRCA2| c.67 + 1G > A (IVS2 + 1G > A) | 2    | Proband (Breast Cancer)     | 65   | Mutated            |
|        |      |          |      | Son (Unaffected)           | 42   | Mutated            |
|        |      |          |      | Son (Unaffected)           | 38   | Mutated            |
|        |      |          |      | Son (Unaffected)           | 37   | Mutated            |
|        |      |          |      | Brother (Unaffected)       | 71   | Mutated            |
|        |      |          |      | Nephew (Unaffected)        | 37   | Wild Type          |
| 18     | BRCA2| c.1238delT (p.Leu413HisfsX17) | 10   | Proband (Breast Cancer)     | 41   | Mutated            |
|        |      |          |      | Son (Unaffected)           | 21   | Mutated            |
|        |      |          |      | Sister (Breast Cancer)      | 24   | Mutated            |
|        |      |          |      | Nephew (Unaffected)        | 24   | Mutated            |
|        |      |          |      | Niece (Unaffected)         | 36   | Mutated            |
|        |      |          |      | Niece (Unaffected)         | 35   | Mutated            |
|        |      |          |      | Cousin (Prostate Cancer)    | 47   | Mutated            |
|        |      |          |      | Cousin (Unaffected)        | 41   | Wild Type          |
| 19     | BRCA2| c.1496_1497delAG (p.Gln499fs) | 10   | Proband (Breast Cancer)     | 41   | Mutated            |
|        |      |          |      | Son (Unaffected)           | 21   | Mutated            |
|        |      |          |      | Daughter (Unaffected)      | 24   | Mutated            |
|        |      |          |      | Sister (Breast Cancer)      | 55   | Mutated            |
|        |      |          |      | Nephew (Unaffected)        | 24   | Mutated            |
|        |      |          |      | Niece (Unaffected)         | 36   | Mutated            |
|        |      |          |      | Niece (Unaffected)         | 35   | Mutated            |
|        |      |          |      | Cousin (Prostate Cancer)    | 47   | Mutated            |
|        |      |          |      | Cousin (Unaffected)        | 41   | Wild Type          |
| 20     | BRCA2| c.4322_4323delAG (p.Glu1441ValfsTer3) | 11   | Proband (Breast Cancer)     | 62   | Mutated            |
|        |      |          |      | Father (Prostate Cancer)    | 73   | Mutated            |
| 21     | BRCA2| c.5796_5797delTA (p.His1932Glnfs) | 11   | Proband (Breast Cancer)     | 72   | Mutated            |
|        |      |          |      | Cousin (Prostate Cancer)    | 72   | Mutated            |
|        |      |          |      | Cousin (Breast Cancer)      | 68   | Wild Type          |
| 22     | BRCA2| c.6037A > T (p.Lys2013Ter)    | 11   | Proband (Ovarian Cancer)    | 66   | Mutated            |
|        |      |          |      | Daughter (Unaffected)      | 28   | Mutated            |
Table 2. *Cont.*

| Family | Gene   | Mutation                        | Exon | Family Members (Diagnosis)          | Age  | Mutational Analysis |
|--------|--------|--------------------------------|------|-------------------------------------|------|---------------------|
| 23     | BRCA2  | c.6450dup (p.Val2151fs)         | 11   | Proband (Breast Cancer)             | 54   | Mutated             |
|        |        |                                |      | Son (Unaffected)                    | 24   | Wild Type           |
|        |        |                                |      | Daughter (Unaffected)               | 21   | Mutated             |
| 24     | BRCA2  | c.6468_6469delTC (p.Gln2157Ilefs) | 11   | Proband (Ovarian and Colon Cancer)  | 62   | Mutated             |
|        |        |                                |      | Daughter (Unaffected)               | 40   | Wild Type           |
| 25     | BRCA2  | c.6468_6469delTC (p.Gln2157Ilefs) | 11   | Proband (Breast Cancer)             | 49   | Mutated             |
|        |        |                                |      | Sister (Ovarian Cancer)             | 53   | Mutated             |
| 26     | BRCA2  | c.6486_6489delACAA (p.Lys2162Asnfs) | 11   | Proband (Breast Cancer)             | 50   | Mutated             |
|        |        |                                |      | Daughter (Unaffected)               | 30   | Mutated             |
|        |        |                                |      | Daughter (Unaffected)               | 28   | Mutated             |
|        |        |                                |      | Sister (Unaffected)                 | 52   | Wild Type           |
|        |        |                                |      | Brother (Unaffected)                | 49   | Mutated             |
| 27     | BRCA2  | c.7007G > A (p.Arg2336His)      | 13   | Proband (Breast Cancer)             | 32   | Mutated             |
|        |        |                                |      | Mother (Unaffected)                 | 57   | Wild type           |
|        |        |                                |      | Father (Prostate Cancer)            | 63   | Mutated             |
|        |        |                                |      | Sister (Unaffected)                 | 27   | Wild type           |
| 28     | BRCA2  | c.7857G > A (p.Trp2619Ter)      | 17   | Proband (Breast Cancer)             | 45   | Mutated             |
|        |        |                                |      | Sister (Unaffected)                 | 48   | Wild Type           |
|        |        |                                |      | Daughter (Unaffected)               | 23   | Mutated             |
|        |        |                                |      | Daughter (Unaffected)               | 22   | Mutated             |
|        |        |                                |      | Son (Unaffected)                    | 76   | Mutated             |
| 29     | BRCA2  | c.8954_8955delGTATinsAA         | 23   | Proband (Colon Cancer)              | 76   | Mutated             |
|        |        |                                |      | Son (Unaffected)                    | 39   | Wild Type           |
|        |        |                                |      | Daughter (Unaffected)               | 44   | Mutated             |
|        |        |                                |      | Father (Breast Cancer)              | 76   | Mutated             |
|        |        |                                |      | Sister (Thyroid Cancer)             | 70   | Mutated             |
|        |        |                                |      | Brother (Prostate Cancer)           | 66   | Mutated             |
|        |        |                                |      | Niece (Unaffected)                  | 44   | Mutated             |
|        |        |                                |      | Niece (Unaffected)                  | 23   | Wild type           |
|        |        |                                |      | Nephew (Unaffected)                 | 42   | Wild type           |
|        |        |                                |      | Nephew (Unaffected)                 | 44   | Mutated             |
| 30     | BRCA2  | c.631G > A (p.Val221Ile)        | 7    | Proband (Breast Cancer)             | 46   | Mutated             |
|        |        |                                |      | Daughter (Unaffected)               | 23   | Mutated             |
|        |        |                                |      | Daughter (Unaffected)               | 22   | Mutated             |
|        |        |                                |      | Daughter (Unaffected)               | 18   | Wild Type           |
|        | BRCA2  | c.7008-2A > T (IVS13-2A > T)   | 14   | Sister (Unaffected)                 | 46   | Mutated             |
|        |        |                                |      | Sister (Unaffected)                 | 42   | Wild Type           |
|        |        |                                |      | Sister (Unaffected)                 | 36   | Wild Type           |
| 31     | PALB2  | c.1919C > A (p.Ser640Ter)       | 5    | Proband (Breast Cancer)             | 30   | Mutated             |
|        |        |                                |      | Father (Prostate Cancer)            | 70   | Mutated             |
| 32     | MUTYH  | c.1437_1439del GGA (p.Glu480del) | 14   | Proband (Breast Cancer)             | 85   | Mutated             |
|        |        |                                |      | Daughter (Prostate Cancer)          | 78   | Mutated             |
|        |        |                                |      | Brother (Endometrial Cancer)        | 55   | Mutated             |
|        |        |                                |      | Son (Unaffected)                    | 58   | Mutated             |
| 33     | RAD51C | c.(705 + 1_706-1)._837 + 1_838-1del | 5    | Proband (Gastric Cancer)            | 58   | Mutated             |
|        |        |                                |      | Cousin (Endometrial Cancer)         | 54   | Mutated             |
| 34     | TP53   | c.817C > T (p.Arg273Cys)        | 8    | Proband (Breast Cancer)             | 46   | Mutated             |
|        |        |                                |      | Daughter (Unaffected)               | 20   | Mutated             |
|        |        |                                |      | Son (Unaffected)                    | 24   | Mutated             |

†: dead.

4. Discussion

The main findings of the present study are as follows. (1) This is one of the few studies focused on Italian patients with hereditary prostate cancer; we have shown pathogenic germline mutations in five susceptibility genes and a monoallelic frameshift mutation in MUTYH, a potentially pathogenic monoallelic mutation whose significance in PCa has not been established. (2) The mutational frequency between BRCA1 and BRCA2 genes was similar, and higher than previously reported. (3) We observed frequent PVs in the 3′ of both BRCA1 and BRCA2, and a large number of PVs in BRCA2 were present in exon 11 of the gene, suggesting a mutation cluster region relating to risk of HPCA. (4) MUTYH
could be related to overall HPCa risk. (5) The mean age of onset in PCa cases that recur in HBOC families was no different in mutated PCa patients versus non-mutated, while the mean survival was highest in mutated patients compared to wild type. (6) PCa is the second most recurrent cancer from our cohort, resulting in 18% of cases in both mutated and non-mutated families.

We identified mutations in 21.6% of HBOC families; mutations in BRCA1 and BRCA2 were found at similar frequency in our cohort. The percentage of mutations found is higher than reported in previous studies that have previously demonstrated that most mutations present were primarily in BRCA2, 1.2–5.3%, while BRCA1 mutations were found in 0.9–1.25% of HPCa patients [16–26]. The difference in mutation prevalence could be influenced by the selection of the patients included; indeed, studies including only HPCa patients report a lower mutation rate than studies including HPCa families. We also identified a family that had DM in both BRCA1 and BRCA2 genes, with a separate family harboring two mutations in BRCA2. These pathogenic variants co-segregate and do not generate a worse phenotype than a single mutation [27].

Patients who were carriers of genes with VUS, where the variants were not classified as either benign or pathogenic, were difficult to interpret in terms of clinical management. The evaluation of their pathogenic significance needs to be corroborated by further experimental evidence. Co-segregation analysis and in silico analysis may be helpful to improve VUS classification [28,29].

Previous studies proposed that mutations in BRCA2 exon 11 were closely related to breast/ovarian cancer risk and aggressiveness [30]. Patel et al. hypothesized that the region between c.7914 and 3’ of the BRCA2 gene is a cluster region associated with an increased risk of developing prostate cancer [31]; however, only one of the mutations found in our patients falls in that region. In the present study, most of the PVs fell in the 3’ of both BRCA1 and in BRCA2 genes and in exon 11 of BRCA2 gene, suggesting a mutation cluster region relating to risk of HPCa. Additional research is expected to confirm this association.

PVs in low-penetrance genes, PALB2, RAD51C and TP53, are present in 0.56% of families. This is in line with previously published studies indicating mutations in other genes in about 0.1–0.2% of cases [19,32]. Despite the rarity of reported PALB2 alterations, recent findings have supported an increasing role of PALB2 in PCa, particularly in metastatic cases [33]. TP53 genetic alterations were reported in 3–12.5% of cases which were considered late events and associated with metastatic castrate-resistant prostate cancer (mCRPC) [34]; however, other studies reported an inactivation of TP53 at a high frequency in primary as well as metastatic castration-naïve PCa [35].

Biallelic MUTYH mutations have been linked to MUTYH-associated polyposis syndrome (MAP). In the past few years, monoallelic MUTYH PVs have been found in patients with gastric, liver, endometrial, breast, ovarian and pancreatic cancer [36,37], and this has led to several studies investigating the impact of germline monoallelic MUTYH PVs in tumorigenesis. We found that 0.56% of families showed a monoallelic frameshift MUTYH PV, while a previous study reported MUTYH PVs in 2.37% of patients with a family history of prostate cancer [38]. Our results suggest an involvement of monoallelic MUTYH mutations in PCa onset; however, additional studies are required.

Mutated and non-mutated patients had a similar mean age of onset, although HPCa showed a trend towards an earlier onset age compared to sporadic cases [39]. No difference has been reported in overall survival between HPCa and sporadic PCa [2]. However, previously, Castro et al. described that BRCA1/2 mutations confer a more aggressive PCa phenotype and lower survival. Additionally, Narod et al. observed a median survival from diagnosis of 4.0 years for men with BRCA2 mutation in antithesis to 8.0 years for men with BRCA1 mutation, a factor that was recommended to be considered for the clinical management of patients [40–42]. We found an average survival of 8 years in PCa patients, with a similar average age of death between mutated and non-mutated. This difference could be due to the size of cohort as well as different clinical aspects of the enrolled patients. BRCA2 germline mutation provided a greater contribution to increased PCa risk compared to BRCA1. Carriers of BRCA2 mutations have an 8.6-fold elevated risk of developing HPCa.
compared to non-carriers; the relative risk of HPCa developing in BRCA1 mutation carriers is increased 3.8-fold [43,44]. BRCA2 pathogenic variants have also been associated with an increased risk of high-grade disease and progression to mCRPC [2]. Here, in HBOC families, PCa occurs in 18% of cancer cases, making it the second most recurrent cancer. The frequency of PCa was equal in both mutated and non-mutated families. Interestingly, there was no significant difference in prostate cancer development between non-mutated HBOC families and HBOC families that harbored mutations in BRCA, PALB2, RAD51C, TP53 and MUTYH genes. This is likely because the categorized “non-mutated” HBOC families could be carriers of pathogenic mutations in genes not yet identified. Screening for mutation in BRCA1 and BRCA2 has the potential to help in the stratification of patients to more targeted therapies. Indeed, patients with mutations in the BRCA genes have a better response to PARP inhibitors. This led the Food and Drug Administration (FDA) to approve the drug Olaparib for patients with mCRPC who harbor mutation in BRCA1/BRCA2 and/or ATM genes. MMR-mutated patients can benefit from immunotherapy, with the FDA having recently approved PD-L1 inhibitor pembrolizumab (KEYTRUDA) for the treatment of patients with microsatellite instability-high (MSI-H)/MMR-deficient [45–47].

Furthermore, clinical trials undertaken with prostate cancer patients who are carriers of a mutant PALB2 have demonstrated a positive response to Olaparib treatments, particularly for patients with resistance to treatment [33,48].

TP53 mutations have been associated with an unfavorable response to antiandrogens, abiraterone and enzalutamide, although this mechanism remains to be elucidated. Whether and to what extent TP53 alterations affect the response to the PARP inhibitor Olaparib is currently unclear [35].

Surveillance should be conducted regularly with PSA assay, urological examination and/or magnetic resonance imaging annually, starting from age 41. In our cohort, the 41 unaffected HBOC family members who inherited the proband’s mutation have an increased risk of developing PCa or other related tumors. The potential consequences relating to tumorigenesis could be reduced through the early detection of the cancer. Therefore, all 41 unaffected HBOC family members who are carriers of the proband’s mutations were enrolled in an active surveillance program for PCa and other related tumors in our hospital. Women with BRCA1, BRCA2, PALB2 and RAD51C mutations were enrolled in a surveillance program for breast, ovarian and colon cancer development, whereas men with BRCA1, BRCA2, PALB2 and RAD51C mutations were enrolled in a surveillance program targeting breast, prostate, colon, laryngeal and pancreatic cancer.

5. Conclusions

In this study, one of the few studies focused on Italian patients with hereditary prostate cancer, we showed pathogenic germline mutations in five susceptibility genes in HBOC probands. In addition, we founded a monoallelic frameshift mutation in MUTYH gene, suggesting an involvement of monoallelic MUTYH mutations in PCa onset. Testing family members of mutated patients gives them the opportunity to optin to surveillance programs for HPCa and other HBOC-related tumors, decreasing the potential consequences relating to cancer development through early detection.

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Institutional Review Board Statement: This study was carried out in accordance with the World Medical Association Helsinki Declaration, adopted in 1964 and amended in 1975, 1983, 1989, 1996 and 2000. Informed consent was obtained from all subjects, and the study was approved and conducted according to the ethical guidelines of the University of Campania “Luigi Vanvitelli” (no. 469-23 July 2019).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.
**Data Availability Statement:** Not applicable.

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