Original Research Article

Clinical Significance of Gene Mutations and Polymorphic Variants and their Association with Prostate Cancer Risk in Polish Men

Cancer Control
Volume 29: 1–12

DOI: 10.1177/10732748211062342
journals.sagepub.com/home/ccx
SAGE

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Abstract

Objectives: We tested the association of germline variants in *BRCA1*, *BRCA2*, *CHEK2*, *CDKN2A*, *CYP1B1*, *HOXB13*, *MLH1*, *NBS1*, *NOD2* and *PALB2* genes, as well as in 8q24 region, with prostate cancer (PC) risk and estimated their impact on disease clinical course, including overall survival time in Polish men with localized PC qualified for radical treatment.

Materials and Methods: DNA of 110 patients with localized prostate cancer treated with radical prostatectomy (RP), from each age group and with different stages of the disease. DNA samples of the control group consisted of 111 men, volunteers, without PC (age-matched to study group). Sanger sequencing, AS-PCR, RFLP-PCR, and multiplex-PCR were used for variants detection.

Results: The percentage of men with ≥1 germline variant was higher in PC group (52.7%) than in healthy men (37.8%) (P = .03). The presence of ≥2 variants was associated with shorter survival than the presence of one or no variant in the PC group (P = .14, trend). The *HOXB13* G84E was detected in 2.9% of PC men and in no healthy men (P = .19, trend, OR = 7.21). A *CHEK2* truncating mutation (1100delC or IVS2+1G>A) was detected in 2/110 (1.8%) PC patients and in no healthy men (P = .29, OR=5.14). The NBS1 I171V was detected in 2/110 (1.8%) PC patients and in no men from the control group (OR=5.14, P = .29, NS).

Conclusions: We conclude that the presence of more than 2 germline variants was probably associated with shorter survival of patients with localized prostate cancer qualified for radical treatment. The *HOXB13* (G84E), *CHEK2* (1100delC or IVS2+1G>A) truncating variants and *NBS1* (I171V) are associated with PC and hereditary form of the disease. The *HOXB13* (G84E) and *NOD2* (3020insC) single variants are associated with shorter and *CYP1B1* (48CC, 119GG) single genotypes with longer overall survival.

Keywords

prostate cancer, germline variants, clinical significance, early detection

Introduction

Prostate cancer (PC) is recognized as one of the most serious problems endangering men's health and life. A significant proportion of PC diagnoses may be associated with a strong hereditary component.[1] The familial clustering of prostate cancer is observed in about 10-20% of patients. Genes of high penetrance may be responsible for 5-10% of prostate cancer cases and as many as 30-40% of early PC onset.[2] Approximately 8% to 12% of patients with advanced disease may be

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the tumor suppressor gene germline variant carriers.[3,4] The numerous single gene germline variants have been confirmed to increase the risk of prostate cancer. These include *BRCA1*, *BRCA2*, *MLH1*, *MSH2*, *MSH6*, *PMS2*, *HOXB13*, *CHEK2*, *NBN*, *BRIP1*, and *ATM* genes.[5,6] However, none of them is a PC high-risk gene. Additionally, more than 70 common low penetrance germline variants increasing PC risk have been identified through Genome Wide Association Studies (GWAS) but their contribution to PC etiology is of relatively low importance.[7-9] The molecular and genetic basis of prostate cancer may involve multiple pathways. One of them may be the formation of double-strand breaks of DNA. However, there are two major pathways of repairing them: homologous recombination and nonhomologous DNA end joining (NHEJ). Efficient and specific repair of DNA damage maintains the genomic integrity of the cell and ensures its ability to persist and proliferate. The platinum based therapies or therapies with PARP (poly[adenosine diphosphate-ribose] polymerase) inhibitors in men with metastatic PC, carriers of known germline variants of DNA damage response (DDR) genes may lead to improved long-term treatment outcomes, although additional research is needed in these areas.[10-12] Multiple large studies have revealed a high prevalence of germline variants in men with advanced prostate cancer.[4,13-16] There is increasing evidence that loss of DNA repair genes function is highly predictive of poor treatment responses.[17]

Prostate cancer is a heterogeneous disease, with a wide spectrum of age at onset and of clinical severity. Radical prostatectomy is the treatment of choice for localized disease. Risk calculators are very well standardized. Ahead of each step of the treatment, many advanced instruments are being used. That includes not only clinical staging and specially dedicated Gleason grade but also many other tools like serum level of PSA (prostate specific antigen), advanced imaging studies such as multiparametric magnetic resonance, bone scintigraphy or even positron emission tomography. Risk scale such as d'Amico classification (which includes Gleason grade, clinical stage and PSA level) helps to determine the general risk of the disease at the diagnosis. Furthermore, many algorithms such as Briganti nomogram and Partin tables are being used to evaluate the risk of metastases. Nevertheless, even after the usage of numerous instruments we are not able to elucidate the causes of most cases of disease recurrence. Therefore, we suppose that the knowledge about hereditary predisposition to prostate cancer in the patients could be helpful to accurately predict their disease recurrence risk after radical prostatectomy.

In the present study, we were looking for 20 known germline variants in *BRCA1, BRCA2, CHEK2, CDKN2A, CYP1B1, HOXB13, MLH1, NBS1, NOD2* and *PALB2* genes, as well as in 8q24 region, in patients with localized prostate cancer treated with radical prostatectomy and in control group. To establish whether these variants contribute to PC development in Polish patients, and to measure their impact on cancer risk, and on the clinical characteristics of the disease, including survival time, we genotyped 110 prostate cancer men and 111 controls. We hypothesized that such germline variants would be more common in patients with higher Gleason score, higher tumor stage, and that carriers of them would have shorter overall survival time compared to non-carriers.

Materials and Methods

The material of the investigations was archival DNA samples extracted in the Department of Clinical Genetics Collegium Medicum Nicolaus Copernicus University in Bydgoszcz between 2005 and 2007. DNA was isolated from EDTA anticoagulated peripheral blood of 110 consecutive, newly diagnosed prostate cancer patients from all over Poland, regardless of age at PC onset, family history and histological type of cancer. They were hospitalized because of PC at Department of Urology of the J. Biziel University Hospital in Bydgoszcz. A prostatectomy was performed in all patients because of primary localized prostate adenocarcinoma.

The age at PC diagnosis ranged from 45 to 75 years (the mean age was 59.9 ± 5.9). A family history was analyzed either by the construction of a family pedigree or the completion of a standardized questionnaire by patients. All cases of first- and second- degree relatives diagnosed with prostate cancer and other cancer types and their age at disease diagnosis were recorded. The estimation of patient families as those with a history suggesting hereditary risk of prostate cancer was performed on the basis of criteria defined by Carter et al.[18] and Cybulski et al.[19] Among 110 prostate cancer patients, 25 (22.7%) originated from families suspected of HPC. In 61 of 110 (55.45%) families, an aggregation of cancers of the breast, stomach, colon, ovary, lung, larynx, bladder and kidney, as well as melanoma was present, in addition to prostate cancer.

Information about the PSA level before the surgical operation was available for 97 patients, about grade of prostate cancer for 101 patients, and about tumor stage for 102 patients.

Data on survival were available for 103 PC patients. The patients were followed from the date of biopsy (confirmation of prostate cancer) until death, if applicable or the end of observation. In living patients fiveyear survival was analyzed.

Control group consisted of DNA samples isolated from peripheral blood of 111 men, volunteers, healthy at the time of the investigations, i.e. without prostate cancer on the basis of PSA concentration and/or digital rectal examination - DRE. The medical examinations were performed in them as a part of PC prophylaxis. The molecular test was offered to all men of control group. The age of men from control group ranged from 46 to 74 years (the mean age was 59.9 ± 6.6) and matched to the PC group. The purpose of the establishing the control group was to estimate with accuracy the frequency of founder variants of known cancer predisposition genes in the Polish population. Men with any cancer diagnosed in a first-degree relative were excluded from the control group.

All consecutive patients with PC qualified for radical treatment, admitted according to the hospitalization list, without any selection were included in the study (all age group and different stages of the disease). However, according to the European Association of Urology Guidelines, only patients with life expectancy of over 10 years are being qualified for radical prostatectomy. Having that in mind, patients that were disqualified from radical treatment for any reason such as numerous comorbidities and with a disseminated neoplastic process or those qualified for systemic treatment were excluded from this study.

The sample size was determined based on Altman's nomogram. A proportion difference of 20% and a test power of 80% were assumed.

The study protocol was approved by the Ethics Committee of the Collegium Medicum Nicolaus Copernicus University in Bydgoszcz, Poland, committee approval number: KB 326/2010. Every PC patient and a men from control group gave their written informed consent for the use of their DNA sample for the genetic testing. Our study was classified as non-interventional prognostic clinical study.

Genotyping

Genomic DNA was isolated from peripheral blood leukocytes by the standard salting-out method. The founder variants in *BRCA1* (4153delA, C61G, 5382insC), *CDKN2A* (A148T), *CHEK2* (1100delC, I157T, IVS2+1G>A, del5395), *CYP1B1* (R48G, A119S, L432V), *NBS1* (657del5, I171V) and *NOD2* (3020insC) were detected as described previously.[20-26] The *HOXB13* G84E, *BRCA2* c.5946delT, *MLH1* A681T, 8q24 rs188140481, *PALB2* c.509_510delGA, c.172_175delTTGT were genotyped using Sanger sequencing method and the primers for amplification of genes coding regions were designed using Primer3 software (http://frodo.wi.mit.edu/) and are available on request. The DNA fragments were sequenced using BigDye terminator v3.1 Cycle Sequencing Kit (Life Technologies), according to manufacturer protocol. Sequencing products were analyzed on the ABI prism 3130 Genetic Analyzer (Applied Biosystems, Life Technologies). All sequences were compared with reference sequences for variant detection (for *HOXB13* NG_033789.1, for *BRCA2* NG_012772.3, for *MLH1* NG_007109.2, for *PALB2* NG_007406.1, and for rs188140481 NC_000008.11).

Table 1. The association of Germline Gene Variants, Including 8q24 rs188140481 with Prostate Cancer Risk.

Gene variant	Controls n/N (%)	PC Patients n/N (%)	OR	95% CI	*P*-Value
CDKN2A A148T	6/111 (5.4)	4/110 (3.6)	.66	.18–2.41	.53
Any *CHEK2* variant	6/111 (5.4)	7/110 (6.4)	1.19	.39–3.66	.76
Any *CHEK2* truncating variant	0/111 (.0)	2/110 (1.8)	5.14	.24–108.27	.29
1100delC	0/111 (.0)	1/110 (.9)	3.05	.12–75.81	.50
IVS2+1G>A	0/111 (.0)	1/110 (.9)	3.05	.12–75.81	.50
CHEK2 1157T missense variant	6/111 (5.4)	5/110 (4.5)	.83	.25–2.82	.77
HOXB13 G84E	0/103 (.0)	3/103 (2.9)	7.21	.37–141.35	.19
Any *NBS1* variant	2/111 (1.8)	4/110 (3.6)	2.06	.37–11.47	.41
657del5	2/111 (1.8)	2/110 (1.8)	1.01	.14–7.30	.99
I171V	0/111 (.0)	2/110 (1.8)	5.14	.24–108.27	.29
NOD2 3020insC	9/111 (8.1)	9/110 (8.2)	1.01	.39–2.65	.98
PALB2 c.172_175delTTGT	0/111 (.0)	1/110 (.9)	3.05	.12–75.81	.50
8q24 rs188140481	0/111 (.0)	1/110 (.9)	3.05	.12–75.81	.50
CYP1B1 R48G (48CG)					
CC	50/109 (45.9)	45/109 (41.3)	.83	.49–1.42	.49
CG	48/109 (44.0)	46/109 (42.2)	.93	.54–1.59	.78
GG	11/109 (10.1)	18/109 (16.5)	1.76	.79–3.93	.17
CC or CG	98/109 (89.9)	91/109 (83.5)	.57	.25–1.27	.17
CYP1B1 A119S (119GT)	51/110 (46.4)	44/110 (40.0)	.77	.45–1.32	.34
GG	48/110 (43.6)	49/110 (44.5)	1.04	.61–1.77	.89
GT	11/110 (10.0)	17/110 (15.5)	1.65	.73–3.70	.23
TT	99/110 (90.0)	93/110 (84.5)	.61	.27–1.3	.23
GG or GT					
CYP1B1 L432V (432CG)	40/109 (36.7)	43/109 (39.4)	1.12	.65–1.94	.68
CC	56/109 (51.4)	45/109 (41.3)	.67	.39–1.14	.14
CG	13/109 (11.9)	21/109 (19.3)	1.76	.83–3.73	.14
GG	96/109 (88.1)	88/109 (80.7)	.57	.27–1.20	.14
CC or CG					

Legend: n— number of variant carriers, N—total number of tested men in the group, OR—odds ratio, CI—confidence interval, $P < .05$.

Statistical Analysis

The prevalence of germline variants in patients vs controls was compared. ORs were generated from two-by-two tables and statistical significance was assessed with the Fisher's exact test or the chi-square test, when appropriate. The ORs were used as estimates of relative risk. Mean age at PC diagnosis was compared between gene variants carriers and non-carriers, using Student's t-test. For the survival analysis, the patients were followed from the date of biopsy (confirmation of prostate cancer) until death or in living patients fiveyear survival was analyzed. The vital status and the date of death were requested from the Polish Ministry of Health. The Kaplan–Meier survival curves for variant carriers and non-carriers have been made. The $P < .05$ was calculated.

Results

At least one variant of a cancer predisposition gene, including 8q24 region was found in 58 of 110 (52.7%) prostate cancer patients and in 42 of 111 (37.8%) men from control group. The difference was statistically significant ($P = .03$). All germline variants detected in patients and control persons are presented in Table 1. The 49 of 58 (84.5%) PC patients, carriers of ≥1 variant, had at least one close relative with other cancer, including breast, uterus, stomach, colon, ovary, lung, larynx, bladder, pancreatic cancer and melanoma or brain tumor.

The *HOXB13* G84E was detected in 3/103 (2.9%) PC men (heterozygous carriers) and in no healthy men. The difference in frequency of G84E between these two groups was not statistically significant ($P = .19$, trend), but with high odds ratio (OR = 7.21). A *CHEK2* truncating variant (1100delC or IVS2+1G>A) was detected in 2/110 (1.8%) prostate cancer patients and in no healthy men. The difference was not statistically significant ($P = .29$), but odds ratio was 5.14. The *NBS1* I171V was detected in 2/110 (1.8%) prostate cancer patients and in no men from control group (OR = 5.14, $P = .29$, NS).

The difference in *CYP1B1* R48G, A119S, and L432V genotypes distribution between prostate cancer patients and healthy controls was not statistically significant, however, more men with PC than healthy men were carriers of abnormal homozygous genotypes. The *CYP1B1* 48GG was detected in 16.5% of PC patients compared to 10.1% of controls, the 119TT in 15.5% of PC patients compared to 10.0% of controls and the 432GG in 19.3% of PC patients compared to 11.9% of controls, respectively: $P = .17$ (trend), $P = .23$ (NS), $P = .14$ (trend). Additionally, a higher frequency of both normal homozygous or heterozygous genotypes for each *CYP1B1* codon was observed in healthy men compared to prostate cancer patients, respectively: 48CC or 48CG (89.9% vs 83.5%, $P = .17$), 119GG or 119GT (90.0% vs 84.5%, $P = .23$), and 432CC or 432CG (88.1% vs 80.7%, $P = .14$, trend). We also found that the 432CG heterozygous genotype was detected with higher frequency in controls than in patients (51.4% vs 41.3%, $P = .14$, trend).

To evaluate the effects on prostate cancer risk, of the three *CYP1B1* SNPs (Single Nucleotide Polymorphisms) in combination, the haplotypes were constructed. Their distribution is shown in Table 2. The together 48CC+119GG+432CC or 48CC+119GG+432CG haplotypes were detected with lower frequency in PC group (19.3%) than in control group (33.9%) and the difference was statistically significant ($P = .02$, OR = .46). The48CC + 119GG + 432CC or 48CC + 119GG + 432CG separately haplotypes analysis showed the higher frequency of both in control than in PC group (respectively: 11.0% vs 4.6% ($P = .09$, trend) and 22.9% vs 14.7% ($P = .12$, trend). The 48GG + 119TT + 432CC haplotype occurred in 17/109 (15.6%) prostate cancer patients and in 11/109 (10.1%) healthy men, but this difference was not statistically significant ($P = .23$).

The *PALB2* c.172_175delTTGT and the 8q24 rs188140481 were found in single patients with PC and in no healthy men.

Table 2. The *CYP1B1* Haplotype Frequency and Prostate Cancer Risk.

CYP1B1							
R48G	A119S	L432V	Controls (N = 109) n (%)	PC Patients (N – 109) n (%)	OR	95% CI	*P*-Value
CC	GG	CC	12 (11.0)	5 (4.6)	.39	.13–1.14	.09
		CG	25 (22.9)	16 (14.7)	.58	.29–1.16	.12
		GG	13 (11.9)	21 (19.3)	1.76	.83–3.73	.14
CC	GG	CC or CG	37 (33.9)	21 (19.3)	.46	.25–.86	.02
CC	GT	CC	0 (.0)	3 (2.8)	7.20	.37–141.02	.19
CG	GG	CC	0 (.0)	1 (.9)	3.03	.12–75.15	.50
CG	GT	CC	17 (15.6)	16 (14.7)	.93	.44–1.95	.85
		CG	31 (28.4)	29 (26.6)	.91	.50–1.65	.77
GG	GT	CC	0 (.0)	1 (.9)	3.03	.12–75.15	.50
GG	TT	CC	11 (10.1)	17 (15.6)	1.65	.73–3.70	.23

Legend: n—number of haplotype carriers, N—total number of tested men in the group, OR—odds ratio, CI—confidence interval, $P < .05$.

The chosen variants of *BRCA1* (4153delA, C61G, 5382insC), *BRCA2* (c.5946delT), *CHEK2* (del5395), *MLH1* (A681T), and *PALB2* (c.509_510delGA), were detected in no man from both groups. Therefore, they were omitted in further analyzes.

At least two genetic variants were detected in 22.7% (25/110) of PC patients compared to 12.61% (14/111) of controls ($P = .05$, on the border of statistical significance). However, no statistically significant differences were observed in frequencies of particular multiple variants combinations between study and the control groups. Detailed results of analysis are available on request.

The association of germline variants with hereditary form of prostate cancer is presented in Table 3. The 2 out of 3 *HOXB13* G84E carriers originated from 25 families fulfilling HPC criteria and one from 78 families without HPC (PC frequency: 8% vs 1.3%, OR = 6.70, $P = .13$, trend). Patients positive for *NBS1* I171V were more likely to have a family history of prostate cancer (8.0%) than those who were negative (.0%) (OR = 18.2, $P = .06$, trend).

We also analyzed frequency of variants in different age groups, dividing patients into 2 groups: those with PC diagnosed at ≤60 years, and with PC diagnosed at >60 years of age. In the group of PC patients, carriers of at least one variant, the disease occurred in 62.07% at the age ≤60 and in 37.93% at > 60 (OR = 2.70, $P = .01$). The *CYP1B1* 432GG genotype was detected in 12/109 (11.0%) PC patients and in 5/109 (4.6%) healthy controls at ≤60 years of age ($P = .09$, trend). The mean age at PC onset of *CYP1B1* 432GG genotype carriers from families with HPC was significantly younger than the mean age of such carriers originating from families without HPC ($P = .0077$). The *NBS1* variant (657del5 or I171V) was detected in 3/110 (2.72%) of prostate cancer patients and in no healthy men ≤60 years of age (OR=7.26, P=.19, trend).

No statistically significant differences in the number of patients with PSA concentration >20 ng/mL, high grade Gleason Score (GS ≥ 8), or advanced TNM stage (T3/4), in association with carrier status were observed. There were neither statistically significant differences in mean serum PSA level between single variant carriers and non-carriers. The analyses were performed for all variants, separately. Their results are available on request.

Data on survival were available from 103 prostate cancer patients. Among them 9 patients died during the first five years from the biopsy date (prostate cancer confirmation) and the disease was the cause of patients death: 1 carrier of *CYP1B1* 432GG, 2 carriers of both *CYP1B1* 48GG and 119TT, 1 carrier of *NOD2* 3020insC, 1 carrier of both *NOD2* 3020insC and *HOXB13* G84E, and 4 PC patients with no variant. The Kaplan–Meier survival curves for overall survival of variant carriers compared to non-carriers are shown in Figure 1. The overall survival was significantly shorter for *HOXB13* G84E (Figure 1A) or *NOD2* 3020insC (Figure 1B) carriers compared to non-carriers (respectively: $P = .08$, $P = .08$, trend). The probability of 5-year survival for the *CYP1B1* 48CC genotype carriers was 97.8% compared to 90.6% for 48CG or 48GG genotypes carriers ($P = .08$, trend) (Figure 1C). The probability of 5-year survival for the *CYP1B1* 119GG genotype carriers was 97.7% compared to 90.9% for 119GT or 119TT genotypes carriers ($P = .10$, trend) (Figure 1D).

The 85.2% of carriers of at least two variants, 95.1% of carriers of 1 variant and 97.6% of non-carriers survived 5 years from the diagnosis of PC. The overall survival was

Table 3. The Association of Gene Variants with Hereditary Prostate Cancer.

Gene Variant	Families with HPC (N = 25) n (%)	Families without HPC (N = 85) n (%)	OR	95% CI	*P*-Value
CDKN2A (A148T)	2 (8.00)	2 (2.35)	3.61	.48–27.03	.21
Any *CHEK2* variant	2 (8.00)	5 (5.88)	1.39	.25–7.65	.70
Any *CHEK2* truncating variant	0 (.00)	2 (2.35)	3.61	.48–27.03	.21
1100delC	0 (.00)	1 (1.18)	1.10	.04–27.96	.95
IVS2+1G/A	0 (.00)	1 (1.18)	1.10	.04–27.96	.95
CHEK2 (I157T) missense variant	2 (8.00)	3 (3.53)	2.38	.37–15.09	.36
CYP1B1 (48GG)	3 (12.00)	$15^{(N=*)}$ (17.86)	.63	.17–2.37	.49
CYP1B1 (119TT)	2 (8.00)	15 (17.65)	.41	.09–1.91	.25
CYP1B1 (432GG)	4 (16.00)	$17^{(N=*)}$ (20.24)	.75	.23–2.48	.64
HOXB13 (G84E)	2 (8.00)	$1^{(N=**)}$ (1.28)	6.70	.58–77.22	.13
Any *NBS1* variant	2 (8.00)	2 (2.35)	3.61	.48–27.03	.21
657del5	0 (.00)	2 (2.35)	.65	.03–14.09	.79
I171V	2 (8.00)	0 (.00)	18.2	.84–392.09	.06
NOD2 (3020insC)	2 (8.00)	7 (8.24)	.97	.19–4.99	.97

Legend: n—number of variant carriers, N—total number of analyzed patients in particular groups, OR—odds ratio, CI—confidence interval, $P < .05$, (*84) —the percentage was related to the number of 84 patients from families without HPC, who were carriers of *CYP1B1* 48GG and 432GG genotype, (**78) —the percentage was related to the number of 78 patients from families without HPC, who were carriers of *HOXB13* G84E variant.

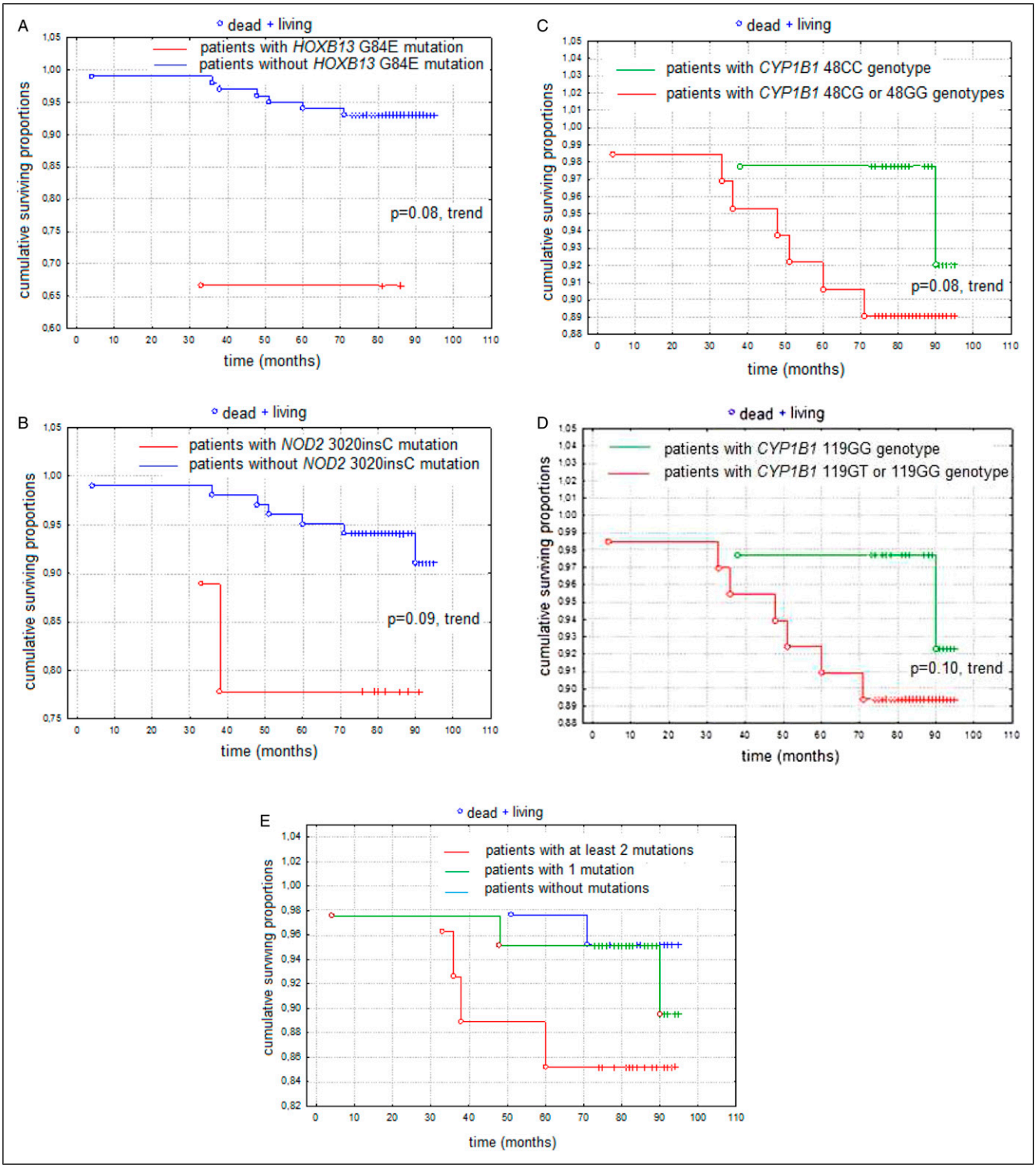

Figure 1. The Kaplan–Meier probability curves for overall survival from PC diagnosis for patients with and without, respectively: (A) *HOXB13* G84E, (B) *NOD2* 3020insC, (C) *CYP1B1* C48G, (D) *CYP1B1* G119T, (E) the Kaplan–Meier probability curves for overall survival from diagnosis of PC for patients with at least 2 variants, with 1 variant, and with no variant in analyzed genes.

wshorter for carriers of at least 2 variants, compared to patients with one or no variant, but the difference was not statistically significant (P = .14, trend) (Figure 1E).

Discussion

Prostate cancer is one of the lifethreatening disorders of male. Genetic susceptibility plays an important role in disease development. In designing strategies for genetic testing, it is important to define the spectrum of pathogenic variants in prostate cancer susceptibility genes. To investigate the frequency of them in Polish patients and to estimate gene-related PC risks and probability of aggressive disease, we analyzed 20 known germline variants in 10 *loci* including 8q24 region in 110 prostate cancer patients and 111 healthy men.

We conclude that the presence of at least one germline variant is an unfavorable factor in PC development. In the group of men up to 60 years of age, the risk of the disease was higher for carriers of at least one variant compared to non-carriers (P = .01). However, the analysis should be confirmed on larger patients groups.

Additionally, we noticed that carriers of at least 2 germline variants had elevated risk of PC and shorter survival time from PC diagnosis than patients with one or no variant. The analysis of the group of PC patients with multiple variants did not lead to finding a characteristic pattern which could be associated with an increased PC risk.

Among 58 prostate cancer patients, carriers of at least 1 germline variant, 49 (84.5%) had at least one close relative with breast, cervix, stomach, colon, ovary, lungs, larynx, bladder, pancreas cancer or melanoma and brain tumor. We suppose that the developing of various types of cancer including PC may be associated with the same hereditary germline variant in different members of the family. In the present study, *HOXB13* G84E carriers had close relatives with colorectal or liver cancers. In Beebe-Dimmer et al. study, G84E was also observed in close relatives of PC patients, who had bladder cancer or leukemia.[27] In our study, among 7 PC patients- carriers of any *CHEK2* variant, 6 had at least one close relative with breast, larynx, colorectal or uterine cancer. This observation is similar to those of others.[21,28] Analysis of the literature indicates that an increased risk of breast, ovarian, colorectal or hematological cancers, as well as melanoma, may be associated with *NBS1* gene variants.[24,29–34] In our study, PC patients, carriers of *NBS1* 657del5 or I171V, had close relatives with breast, colorectal or liver cancers.

In the present study, the *CYP1B*1 R48G (48GG), A119S (119TT) or L432V (432GG) carriers had close relatives with cancers of breast, uterus, stomach, colon, ovary, lung, larynx, bladder and pancreas or melanoma. Lubiński et al. described an increased risk of breast cancer in carriers of all three *CYP1B1* germline variants.[35] In Wang et al. study, the R48G and L432V, but not A119S were associated with a higher risk of endometrial cancer.[36] In Matyjasik et al. study, women who were homozygous for the *CYP1B1* GTC haplotype were at increased risk of breast cancer compared to women with the most common CGG haplotype.[37] The results of the study performed by Sasaki et al. demonstrate that the frequencies of 119TT and 432GG were significantly higher in renal cell cancer patients compared to their frequencies in healthy controls.[38]

In our study, breast cancer was diagnosed in a close relative of PC patient, the *PALB2* c.172_175del carrier. Cybulski et al. estimated the odds ratio for the risk of breast cancer in this variant carriers as 5.02.[39] In Myszka et al. study, the *PALB2* c.172_175del was detected in a woman diagnosed with papillary serous ovarian cancer. She had a family history of cancer that included lung, liver, mouth and gastric cancers.[40]

The *NOD2* 3020insC carriers, described here by us, originated from families without aggregation of cancers other than prostate cancer. However, Lener et al. indicated the association on 3020insC with breast and lung cancer.[41] Kurzawski et al. with colorectal cancer[42] and Liu et al. with colorectal, gastric, breast, lung and laryngeal cancer as well as with lymphoma.[43]

PC patient, *CDKN2A* A148T carrier, reported here had mother with kidney cancer and brother with liver cancer. Studying also Polish population, Dębniak et al. indicated the variant association with melanoma, breast and lung cancer.[23]

In our study, rectal cancer was diagnosed in a relative of PC patient, 8q24 rs 188140481 carrier. The analysis of the available literature indicates that rs188140481 was studied only in relation to prostate cancer risk.[44,45] Because of this variant low frequency in all studies performed until now, larger analyzes are needed to explain its prognostic significance and association with cancer types other than prostate cancer.

An interesting finding of our study is the correlation between *HOXB13* G84E germline variant and predisposition to PC and hereditary form of the disease. Additionally, G84E carriers have shorter overall survival and probably older age at PC onset compared to non-carriers. In our study, the variant was present in three men with prostate cancer (2.9%) and in no healthy man. In many recent studies, *HOXB13* G84E also was found with higher frequency in PC men than in the control group.[46-50] In the present study, the odds ratio (OR) of PC occurrence in G84E carriers was estimated at 7.21. A meta-analysis of 20 publications of available evidence on *HOXB13* G84E and PC risk to date, performed by Nyberg et al. revealed significant heterogeneity between reported relative risks (OR range: .95–33.0) However, except for Chinese population, where the G84E was not present, all studies carried out till now confirmed the significant role of G84E in PC development.[51,52] In our study, the *HOXB13* G84E frequency in patients from families with HPC (8.0%) was significantly higher than in patients from families without HPC (1.3%). The association of G84E with hereditary form of the disease was also found by Breyer et al., Kote-Jarai et al., Kluźniak et al., and Ewing et al.[9,47,49,53] Moreover, we observed that in 2 of 3 G84E carriers the disease was diagnosed at above 60 years of

age (61 and 67 years). On the contrary, some studies reported a younger age at PC diagnosis in G84E carriers compared to non-carriers.[48,53,54] Additionally, Nyberg et al. indicated that the PC risk by age 85 for male *HOXB13* G84E carriers varied from 60% for those with no PC family history to 98% for those with two relatives diagnosed at young ages (≤50), compared with an average risk of 15% for non-carriers.[52] Thus, further analysis of age at PC onset of G84E carriers in Polish population is needed. In our study, two of three (66%) variant carriers and 93% of variant non-carriers survived five years after prostate cancer diagnosis ($P = .08$). The difference between the G84E carriers and non-carriers survival was not observed by Kluźniak et al., who also studied Polish population. However, they attributed it to a small number of persons in the analyzed groups.[49] In Kote-Jarai et al. study, *HOXB13* G84E was neither related to overall survival of PC patients.[9]

In our study, among all tested *CHEK2* pathogenic variants, the most interesting findings were observed for 1100delC or IVS2+1G>A. Carrying any of them was associated with 5-fold higher risk of PC. In Cybulski et al. study, also in Polish population, 1100delC and IVS2+1G>A were detected with higher frequency in 690 prostate cancer patients group (1.6%) than in three times larger control group of 1921 people (.5%) (OR = 3.4, $P = .004$). The association between different germline *CHEK2* pathogenic or likely pathogenic variants and PC risk has been found by Wu et al.[55] The connection between familial PC and *CHEK2* truncating variants in Polish population was found by Cybulski et al. in 2004, by the same researchers in larger patients groups in 2013 and by Wokołorczyk et al. in 2020.[56-58] The association between 1100delC and additionally I157T with HPC was found by Seppala et al. who studied Finnish population.[59] In Polish population Cybulski et al. showed a 16% frequency of *CHEK* I157T in patients from HPC families compared to the general population risk (OR = 3.8, $P = 00002$).[56] In our study the I157T frequency was higher in PC patients from HPC families (8.0%) than in patients from families without HPC (3.5%). The difference in *CHEK2* I157T prevalence between our and Cybulski et al. Polish groups studied (8% vs 16%) may result from different sizes of these groups (110 and 690 patients). The results similar to those found in our study, were obtained by Dong et al., who analyzed a group of 698 PC men from the US population. The difference in frequency of I157T between three groups of persons: with sporadic (1.5%) or familial prostate cancer (2.3%) and men from the control group (1.2%) was not statistically significant.[60] The groups examined by Cybulski et al. and Dong et al. were similar in sizes but results obtained by them were different. It could probable result from the geographical and population differences in I157T occurrence. In the present study, because of low *CHEK2* 1100delC or IVS2+1G>A frequency in PC group (both .9%), it is difficult to conclude about their association with age at disease onset. Studying also Polish population, Cybulski et al. indicated that frequency of any *CHEK2* variant: 1100delC, I157T, IVS2+1G>A, and del5395 was higher (12.4%), in men with PC diagnosed ≤60 years of age compared to analogical frequency in control group (5.8%).[57] There is some controversy whether *CHEK2* predisposes to more aggressive PC. The present and the mostof other studies suggest that *CHEK2*related tumors are similar to those in the population at large.[55,57,58,61] All PC patients, carriers of any tested *CHEK2* pathogenic variants, survived five years from the disease diagnosis, therefore we may conclude that the presence of these variants is probably not associated with shorter overall survival. In Cybulski et al. study, the probability of 5-year survival in group of men with any *CHEK2* variant (71%) was similar to that of non-carriers (72%) (HR = .99, $P = .95$).[57]

In our study, the most important findings of concerning *NBS1* is the association of I171V variant with elevated risk of PC and hereditary form of the disease. Additionally, it seems that *NBS1* I171V or 657del5 founder variants may be associated with younger age at PC diagnosis (≤60). Noteworthy is a high OR of PC occurrence in *NBS1* I171V carrier (5.14). However, the 657del5 appears not to be associated with PC elevated risk because its frequency was the same in patients and controls (1.8%). The relationship between *NBS1* 657del5 and the risk of PC remains controversial. Different studies show conflicting results. Some of them confirm such association, including study results presented by Cybulski et al. on larger Polish patients and control groups,[34,57] but some of them did not confirm such relationship.[32,62] In all studies presented above, the different populations were analyzed; in the present and Cybulski et al. studies, Polish population, in Abele et al. Latvian population, in Hebbring et al. Spanish, African-American, Asian and Caucasian populations with 27 Slavic patients and no people of Slavic origin in the control group. Thus, the difference in the prevalence of *NBS1* 657del5 may result from an inter-populational differences. In our study, two (8.0%) the *NBS1* I171V carriers originated from families with HPC and no one from families without HPC (OR = 18.2, $P = .06$, trend). However, *NBS1* 657del5 was not associated with hereditary form of the disease. In Cybulski et al. study, the *NBS1* 657del5 was present in 9% of probands from families with familial prostate cancer (proband with one or more first- or second-degree relatives with PC) compared to .6% of controls (OR = 16, $P < .0001$).[20] Our results have not confirmed this observation; however it may result from smaller size of examined group in our study. On the basis of present study, it seems that *NBS1* 657del5 may be associated with younger age at PC development (≤60). Cybulski et al. presented similar conclusions.[20] In our study 657del5 and I171V were not more frequent among patients with the aggressive versus nonaggressive phenotype of PC. However, Mijuskovic et al., Wokołorczyk et al., and Cybulski et al., reported opposite results.[57,58,61,63] In our study we did not observe the association between the presence of 657del5 and I171V and overall survival. On the contrary, in Cybulski et al. study, the shorter survival for 657del5 carriers compared to non-carriers was apparent in the first 5 years after diagnosis (HR = 2.08, $P = .002$). The authors observed that the 5-year

survival was achieved by 49% of this variant carriers, compared to 72% of non-carrier controls. Similar results were obtained by Rusak et al.[57,64]

In our study, *CYP1B1* 119TT, 432GG and 48GG homozygous genotypes and 48GG+119TT+432CC haplotype were associated with PC risk. Their frequencies in the PC group (15.5%, 19.3%, 16.5%, 15.6%, respectively) were higher than in control group (10.0%, 11.9%, 10.1%, 10.1%, respectively). Analyzing larger Polish patient and controls groups, Matyjasik et al. indicated 2.2-fold higher frequency of PC in *CYP1B1* 48GG + 119TT + 432CC haplotype carriers compared to non-carriers (13.7% vs 6.6%).[37] In 2013, Zhang et al. performed the meta-analysis of 14 independent case-control studies, including 6380 PC patients and 5807 controls. Authors suggested that *CYP1B1* 119G>T (rs1056827), 432C>G (rs1056836) and 453A>G (rs1800440) variants might be the risk factors for PC. The association of *CYP1B1* 48C>G (rs10012) with PC risk was not confirmed by them.[65] Another meta-analysis performed in 2019 by Zhu et al. did not confirm the association of *CYP1B1* rs10012, rs1800440, rs2551188 (intron variant) and rs162549 (upstream transcript variant) with PC risk.[66] The *CYP1B1* 432C>G neither was associated with PC risk in Caucasian populations.[67] We suppose that *CYP1B1* homozygous (119GG) or heterozygous (119GT) genotypes may be protective in relation to PC development; the frequency of any of them was lower, by 5.5%, in PC patients compared to their frequency in controls. Similar relationships were observed for other *CYP1B1* genotypes and their constructed haplotypes, 48CC and 432CC, 48CG and 432CG, 48CC + 119GG + 432CC or 48CC + 119GG + 432CG. Matyjasik et al. did not found a protective effect of these haplotypes against prostate cancerogenesis.[37] Beuten et al. indicated that common haplotype CC (upstream transcript variant; rs2567206) + GG (intron variant; rs2551188) + CC (intron variant; rs2617266) + CC (codon 48; rs10012) + GG (codon 432; rs1056836) + AA (codon 453; rs1800440) is inversely associated with PC risk in Hispanic Caucasians and with aggressive disease status in nonHispanic Caucasian patients. They also found that a second major haplotype of the above regions, TT+AA+TT+GG+CC+AA, was positively associated with high-grade disease in non-Hispanic Caucasians.[68] Association of 119TT+432CC diplotypes with aggressiveness was also found by Cicek et al.[69] On the contrary, Kochakova et al. did not observe an association between the haplotype combinations of 453, 449, 432 and 119 codons and disease aggressiveness, but they found statistically significant association of the haplotypes: 449TT + 432GG + 119TT (P = .043) and 449TT + 119TT (P = .019) with decreased PC risk in Bulgarian PC patients.[70]

In the present study the 48GG genotype frequency was 7-fold higher in patients from families with two PC cases but not fulfilling HPC criteria than in group of families with sporadic prostate cancer (P = .05). We suppose that the 48GG was associated with familial but not hereditary prostate cancer. Similar results were obtained for 119TT genotype and GTC haplotype (P = .047, P = .047, respectively).

In our study, the mean age at PC onset of *CYP1B1* 432GG genotype carriers from families with HPC (52.5 ± 5.4) was lower, by 8 years, than the mean age of this genotype carriers from families without HPC (60.9 ± 5.0) (P = .0077). Thus, we may assume that 432GG homozygous genotype is associated with younger age at prostate cancer diagnosis (≤60).

Based on the Kaplan–Meier curves analysis, we suppose that men with 48CC or 119GG normal homozygous genotypes have longer overall survival compared to 48GG or 48CG and 119TT or 119GT genotype carriers (P = .08 and P = .10, respectively, trend). The difference between overall survival of 432GG homozygous genotype carriers and normal homozygous (432CC) or heterozygous (432CG) genotype carriers was not statistically significant. However, Gu et al. showed that PC patients, *CYP1B1* 432CC carriers, had higher risk of recurrence in localized prostate cancer (P = .001) compared to 432GG or CG carriers.[71]

In the present study, *NOD2* 3020insC was not associated with PC, it occurred with almost same frequency in prostate cancer patients and controls (8.2% and 8.1%, respectively). This observation is consistent with the results obtained by Lubiński et al. and Liu et al.[43,72] However, in our study, the probability of 5-year survival was lower, by 17.2%, for *NOD2* 3020insC carriers (77.8%) compared to non-carriers (95.0%) (P = .09, trend). The 22.2% of variant carriers and 5.0% of non-carriers died during the first 5 years from the prostate cancer diagnosis. The above should be confirmed in larger patients groups and other populations.

In the present study, the *CDKN2A* A148T was not associated with PC, variant occurred with higher frequency in controls than in prostate cancer patients (5.4% vs 3.6%, respectively), without statistical significance (P = .53). Similar conclusions were presented by Dębniak et al. who also studied Polish population.[23] In our study, the A148T was detected about 3.3 times more frequently (8%) among patients from families with HPC than among patients from families without HPC (2.4%). The difference in the A148T incidence between these groups was not statistically significant. We suppose that this variant may be associated with hereditary prostate cancer, but this observation should be confirmed on larger PC groups. In the present study, the disease was diagnosed in only one A148T carrier at 49 years of age. Such early age at disease onset may indicate a high penetration of this mutation, however more extensive studies are necessary to confirm these findings.

In our study *PALB2* tested pathogenic variants were not associated with PC. However, we cannot rule out that they contribute to PC susceptibility in individual patients. Recent years studies indicated that the metastatic castration-resistant PC patients (mCRPC) with DNA damage response mutations (DDR), including *PALB2,* who are treated with PARP inhibitors or immunotherapies may achieve positive therapeutic responses.[73]

In the present study, the 8q24 rs188140481 variant did not contribute to PC development, it was detected in one PC patient and in no healthy men. Studying also Polish population, Antczak et al. showed that it may confer a moderate increase in the PC risk.[44] Studying North American population, Grin et al. indicated that rs188140481 confers greater risk of PC compared with other SNPs identified by genome-wide association studies.[45] Because of low frequency of rs188140481 in all available studies, larger analyzes are needed to validate the prognostic significance of this *locus,* and its associations with adverse phenomenon.

Our study has of course some limitations. The first of them is the analysis of only chosen 20 germline variants of 10 known cancer predisposition genes. Currently, NGS technologies allow for genotyping of the whole sequences of many genes in order to know the individual predisposition to cancer. Thus, the approach focused on studying single genes variants has been ceased. However, the aim of our study was to check the frequency of germline variants of known cancer predisposition genes, to measure their impact on cancer risk, and on the clinical characteristics of the disease, including survival time, in good prognosis patients qualified for radical treatment.

The second limitation may be unequal impact of studied variants on gene function. Therefore, we decided to perform an additional analysis on the association of each variant with prostate cancer risk, separately.

Another limitation may be the number of men included in our study (110 in the study group and 111 in the control group). However, the sample size was determined based on Altman's nomogram and it is accepted for such analysis.

Conclusion. Our results confirm the evidence that germline constitutional variants of known cancer predisposition genes, especially *HOXB13*, *CHEK2*, and *NBS1* are associated with prostate cancer and clinical features of the disease. The *HOXB13* (G84E) and *NOD2* (3020insC) are associated with shorter and *CYP1B1* (48CC or 119GG) with longer survival of good prognosis prostate cancer patients with localized disease, qualified for radical treatment. Additionally, we noticed that carriers of at least 2 germline variants had an elevated risk of PC and shorter survival from PC diagnosis than patients with one or no variant. In the present study, germline variants were not more common in patients with higher Gleason score and higher tumor stage. We also noted that multiorgan cancer aggregation in a family, including prostate cancer aggregation in close relatives and young age at PC onset in a family should be taken under consideration by clinicians as an indication to refer persons to molecular testing. We are convinced that in the near future the genetic testing will be a key factor in the selection of a specific prostate cancer patient groups from whole group of good prognosis patients with localised PC qualified for radical treatment, in whom the disease may relapse within the first 5 years after PC diagnosis. In the urologist's point of view, this is a very important conclusion to the study that will be continued in our next research and developed throughout the next years.

Acknowledgments

We thank all patients for participating in this study.

Author's Note

I confirm that this work is original and has not been published elsewhere, nor is it currently under consideration for publication elsewhere.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was supported by the fund of the Collegium Medicum Nicolaus Copernicus University, Bydgoszcz, Poland.

Ethical Approval

The study protocol was approved by the Ethics Committee of the Collegium Medicum Nicolaus Copernicus University in Bydgoszcz, Poland, committee approval number: KB 326/2010. Every prostate cancer patient and men from control group gave their written informed consent for the use of their DNA sample for genetic testing.

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