Original paper

G84E germline mutation in *HOXB13* gene is associated with increased prostate cancer risk in Polish men

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We tested the association between *HOXB13* G84E (rs138213197) germline mutation and PC risk in Polish men.

DNA from 103 consecutive, newly diagnosed patients hospitalised because of PC and DNA from 103 men: volunteers, healthy at the time of the study.

The G84E mutation was genotyped using Sanger sequencing.

The *HOXB13* G84E germline mutation was detected in 2.9% of PC men (3/103) and not detected in any healthy man. Two mutation carriers originated from two of 25 families fulfilling hereditary prostate cancer criteria (HPC) and one mutation carrier from one family among 78 families without HPC (PC frequency: 8% vs. 1.3%, OR = 6.70, p = 0.13). In two of three mutation carriers, disease was detected above 60 years of age. There was a trend for a lower probability of 5-year survival in patients with G84E than in patients without it (66.7% vs. 94.0%, p = 0.08).

The HOXB13 G84E germline mutation is associated with increased prostate cancer risk in Polish men, with hereditary form of the disease, and probably with older age at PC onset (> 60 years of age) and shorter survival. However, it is not associated with PSA level, or PC stage or grade at the time of diagnosis.

Key words: prostate cancer, *HOXB13* G84E mutation, hereditary predisposition, clinical characteristics.

Introduction

Prostate cancer (PC) is a serious problem endangering men's health and life. Among all cancers in men it is the most common type in developed countries. In Poland, prostate cancer is the second most common cancer type, after lung cancer [1, 2, 3, 4]. Positive family history of PC remains among the strongest known risk factors for the disease development. The genetic basis of prostate cancer is very complex. Through linkage analysis, numerous prostate cancer chromosomal loci have been identified, including HPC1 (1q25-25), PCaP (1q42-43), HPCX (Xq27-28), CAPB (1p36), HPC20 (20q13), 17p12, and 8p22-23. Three candidate susceptibility genes have been positionally cloned, *RNASEL* in HPC1, *HPC2/ELAC2* in 17p12, and *MSR1* in the 8p22-23 region, but none of these is a high-risk prostate cancer susceptibility gene [5, 6, 7, 8, 9, 10, 11]. Literature analysis indicates that mutations of genes that play important role in cellular cycle regulation and DNA repair mechanisms are associated with higher risk of PC. The *BRCA1*, *BRCA2, CHEK2*, and *NBS1* belong to these groups of genes, but their con-

tribution to prostate cancer aetiology is of relatively low importance [12 13, 14, 15, 16]. More than 70 common low penetrance variants that increase PC risk have been identified to date through Genome Wide Association Studies (GWAS) [17, 18, 19].

In 2012 Ewing *et al.* described for the first time the relationship between G84E (c.251G>A, p.Gly84Glu) germline mutation of the *HOXB13* gene and increased prostate cancer risk. In that investigation the researchers sequenced 202 genes in a prostate cancer linkage region at 17q21.32 among 94 probands from prostate cancer families and they found this rare recurrent mutation in four families. The G84E was found in 1.4% of cases and in only 0.1% of control subjects (OR = 20.1; p < 0.0001) [20]. The G84E is located in the first exon of the gene causing the alteration from glycine to glutamic acid, which is predicted to affect protein function. The normal homeobox protein HOXB13 is involved in embryonic development of different organs and regulates transcription of androgen receptor (AR) target genes that are known to play a role in prostate cancer growth [21 22, 23, 24, 25]. The results obtained by Ewing *et al.* were confirmed by other researchers in different scientific centres, and also indicated that G84E mutation was strongly associated with prostate cancer pathogenesis [19, 26, 27, 28, 29]. In 2012 Breyer *et al.* indicated a 7.9-fold higher risk of prostate cancer in G84E carriers than the population risk [26]. The carrier rate was 1.9% among all familial case probands, 2.7% among probands of pedigrees with at least three affected, and 1.5% among probands with no additional family history of prostate cancer. In 2013, Stott-Miller *et al.* reported results from a population-based, case-control study of men in western Washington State, where they observed the G84E mutation in 1.3% of patients versus 0.4% of controls, which reflects a 3.3-fold higher risk of PC in G84E carriers versus non-carriers [28]. Also in 2013, Kluźniak *et al.* analysing a Polish population indicated at five-fold higher risk of PC in patients with G84E than the population risk [27]. Mutation was found in 20/3515 (0.6%) PC men and in 3/2604 (0.1%) men from the general Polish population. Furthermore, G84E was found in 4/416 (1%) men coming from HPC families, which was associated with a 8.4-fold higher risk of this cancer compared to the general population. In 2014, Karlsson *et al.* examined 5003 cases and 4693 controls from Sweden and showed that the G84E mutation was present in 4.6% of patients and in 1.3% of controls, proving a strong association of this mutation with PC risk (OR = 3.4) [29]. Interestingly, the G84E mutation has not been found among PC patients of Asian ancestry, who instead show other *HOXB13* mutations evidencing allelic heterogeneity in different ethnic backgrounds [30].

To establish whether or not the *HOXB13* G84E mutation contributes to prostate cancer in Poland, and to measure the impact of this mutation on cancer risk, and on the clinical characteristics of prostate cancer, including survival time, we genotyped 103 prostate cancer men and 103 controls.

Material and methods

The material of investigations were archival DNA samples stored in the Department of Clinical Genetics CM UMK in Bydgoszcz from 2005 to 2007. DNA was isolated from peripheral blood of 103 consecutive, newly diagnosed patients from all over Poland, regardless of age at prostate cancer onset, family history, and histological type of cancer. They were hospitalised because of prostate cancer at the Department of Urology of the Jan Biziel University Hospital in Bydgoszcz. The age at prostate cancer diagnosis ranged from 45 to 75 years (the mean age 59.9 ±5.9 years). A family history was taken either by the construction of a family tree or the completion of a standardised questionnaire. All first- and second-degree relatives diagnosed with prostate cancer and the age of diagnosis were recorded. The estimation of patient families as those with a history suggesting hereditary risk of prostate cancer (HPC) was performed on the basis of criteria defined by Carter *et al.* [31] and Cybulski *et al.* [11]. Among 103 prostate cancer patients, 25 (24.27%) originated from families suspected of having Hereditary Prostate Cancer.

Information about the PSA level before the operation was recorded for 97 patients, about the grade of prostate cancer cells for 101 patients, and about tumour stage for 102 patients.

Data on survival were available for 103 PC patients.

In 57 of 103 (55.34%) families, an aggregation of cancers of the breast, stomach, colon, ovary, lung, larynx, bladder, kidney, or melanoma occurred, in addition to prostate cancer.

The control group consisted of DNA samples isolated from peripheral blood of 103 men – volunteers, healthy at the time of the investigations. The age of men ranged from 46 to 74 years (the mean age was 59.9 ±6.6 years; the age of controls was matched to the PC group).

The study protocol was approved by the Ethics Committee of the Collegium Medicum Nicolaus Copernicus University in Bydgoszcz, Poland. Every PC patient or control gave his written, informed consent for the use of DNA samples for genetic testing.

Analysis of G84E mutation

Genomic DNA was extracted from 5-10 ml of peripheral blood by the standard salting-out method. The G84E mutation was genotyped using Sanger sequencing. The primers for amplification of the coding region

of the first exon were designed using Primer3 software (http://frodo.wi.mit.edu/). The primer sequences were: 5'- CTTGGATGGAGCCAAGGATA -3' (HOXB13 Forward) and 5'- CCTCACCAGCTCCAAGTCTC -3' (HOXB13 Reverse). The amplified DNA amplicon was sequenced using BigDye terminator v3.1 Cycle Sequencing Kit (Life Technologies), according to the manufacturer's protocol. Sequencing products were analysed on an ABI prism 3130 Genetic Analyzer (Applied Biosystems, Life Technologies). All sequences were compared with the HOXB13 RefSeq sequence (NG_033789.1) for mutation detection. Sequencing analysis of the fragment of HOXB13 first exon was performed in all men from study and control groups.

Statistical analysis

The prevalence of the mutation in cases and 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 of PC diagnosis was compared between G84E 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. In living patients five-year survival was analysed. The vital status and the date of death were requested from the Polish Ministry of Health. The Kaplan-Meier curves of the survival for *HOXB13* G84E carriers and non-carriers were made.

Results

In our study, the G84E mutation was detected in 3/103 (2.9%) PC men (heterozygous carriers) but not in any healthy man. The difference in frequency of G84E mutation between these two groups was not statistically significant ($p = 0.19$, trend) but with a high odds ratio (OR = 7.21).

Patients positive for G84E mutation were more likely to have a family history of prostate cancer (2/3) than those who were negative (23/100) (66.7% vs. 23%), but this difference was not statistically significant ($p = 0.26$). The pedigrees of the three G84E mutation carriers are shown below (Fig. 1). In the first HPC family, PC was diagnosed in a proband at 51 years of age and in his father at 68 years of age. Additionally, stomach cancer and brain tumour were diagnosed in two of the second-degree relatives of the proband. In the second HPC family, PC was diagnosed in three relatives, in a proband at 67 years of age and in his two uncles (mother's brothers) at unknown age. In this family, stomach cancer was diagnosed in the proband's father at 60 years of age. In the third family, which did not meet the HPC criteria, PC was diagnosed in the proband at 61 years of age and testis cancer in his son at 31 years of age. In all three families the G84E mutation was not tested in probands' relatives.

The G84E carriers were not more likely to be diagnosed with prostate cancer in younger age than non-carriers. The mean age of PC diagnosis was 59.7 ±8.01 years for mutation carriers versus 60.0 ±6.0 years for non-carriers; this difference was not statistically significant ($p = 0.93$). G84E was detected in two of three mutation carriers at more than 60 years of age and in one at less than 60 years.

The clinical characteristics of prostate cancer in G84E carriers and non-carriers are presented in Table I. No statistically significant differences in the presence of PSA concentration >20 ng/ml, high-grade Gleason Score (GS ≥ 8), or advanced TNM stage (T3/4), depending on carrier status, were observed. There were no statistically significant differences between mean serum PSA level in G84E carriers and non-carriers (10.4 vs. 8.1, respectively; $p = 0.12$).

Data on survival were available for all 103 investigated men. There was one death recorded among three G84E carriers and seven deaths among 100 G84E non-carriers. The probability of 5-year survival for the carriers was 66.7%, compared to 94.0% for non-carriers ($p = 0.08$). Figure 2 shows the Kaplan-Meier curves for G84E carriers and non-carriers.

Discussion

The results of our study confirm the recurrent nature of *HOXB13* G84E germline mutation in Polish prostate cancer patients. G84E mutation occurred in three men in the study group (2.9%) but in nobody in the control group. Kluźniak et al., studying a Polish population, also found a higher incidence of G84E mutation in men with PC (0.6%) than in their control group (0.1%). The difference in the prevalence of this mutation in both groups might result from the different sizes of these groups (103 and 2604 persons) [27]. Karlsson et al. showed a higher incidence of G84E in a group of PC men from Sweden than in a national control population collected between 2001 and 2003 (4.6% vs. 1.4%), or in a group of PC men coming only from Stockholm (biopsy-positive patients) than in biopsy-negative controls collected between 2005 and 2007 (4.3% vs. 1.3%) [29]. In this study, the odds ratio (OR) of PC occurrence in G84E carriers was estimated at 7.21 (similarly, in the Breyer et al. study – 7.9, in the Akbari et al. study – 5.8, and in the Storebjerg et al. study – 5.1) [21, 26, 29, 32]. In the study by Kote-Jarai et al. the OR of a PC occurrence in G84E carriers was slightly lower and was estimated at 2.93 (in the Chen et al. study – 3.8) [19, 33]. Except in the Chinese population, where
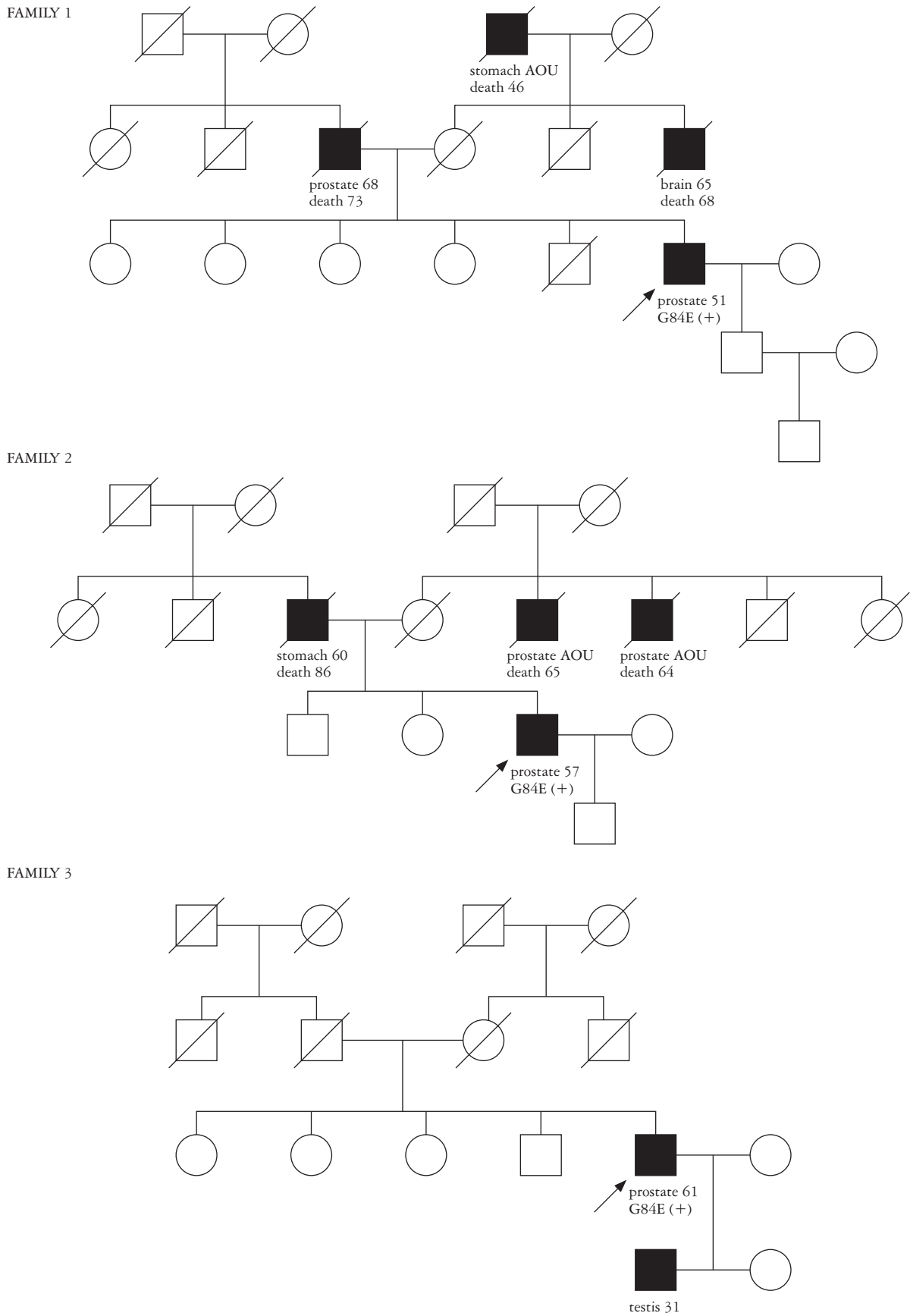
Fig. 1. The pedigrees of three G84E mutation carriers. In each family, the proband is indicated by an arrow. The type of cancer and the age at disease diagnosis are described under the filled black square. G84E(+) means the presence of mutation. AOU means age at onset unknown

Table I. Clinical and laboratory characteristics of prostate cancer in G84E carriers and non-carriers

Factor	G84E carriers number (%)	G84E non-carriers number (%)	P
PSA before the operation (ng/ml)	n = 3	n = 94	
mean PSA	10.4 (7.4-13.4)	8.1 (0.5-53.5)	0.12
≤ 4	0 (0.0)	5 (5.3)	0.7
4.1-10.0	3 (100.0)	59 (62.8)	0.5
10.1-20.0	0 (0.0)	24 (25.5)	0.7
> 20	0 (0.0)	6 (6.4)	0.7
Gleason Score	n = 3	n = 98	
2-4	0 (0.0)	8 (8.2)	0.6
5-7	3 (100.0)	78 (79.6)	0.9
8-10	0 (0.0)	12 (12.2)	0.5
TNM	n = 3	n = 99	
T1-T2	3 (100.0)	83 (83.8)	0.4
T3-T4	0 (0.0)	16 (16.2)	0.4

PSA – prostate-specific antigen; p-values for mutation carriers were calculated with respect to non-carriers as reference group (p < 0.05); TNM – tumour node metastases (T1-T4); Gleason scoring system measures histological grade (2-10) of tumour

the G84E was not present, all the studies carried thus far have confirmed the significant role of G84E mutation in prostate cancer development [20, 21, 26, 27, 29, 30].

Breyer *et al.* revealed a 1.9% G84E frequency in men from families with at least one case of prostate cancer, 2.7% in men from families with at least three PC cases, and 1.5% in men from families in which no PC case was detected [26]. Kote-Jarai *et al.* reported the prostate cancer risk being significantly higher for *HOXB13* G84E mutation carriers with a family history of PC (defined as prostate cancer in a first- or second-degree relative at any age) compared with non-carriers, with an OR of 4.7. The frequency of G84E mutation in prostate cancer patients with family history of PC was 2.4% and in those with no family history (defined as lack of PC in a first- or second-degree relative) was two-times lower, at 1.2% [19]. In our study, the G84E frequency in patients from families with HPC (8.0%) was significantly higher than in patients from families which did not fulfil the HPC criteria (1.3%). The association of G84E with the hereditary form of PC was also confirmed by Kluźniak *et al.* and Ewing *et al.* [20, 27].

An analysis of the age at PC onset of G84E carriers in our study shows that the presence of this mutation may be associated with older age at PC onset; in 2 of 3 G84E carriers, prostate cancer was diagnosed at age > 60 years (61 and 67 years). There was no statistically significant difference in the mean age at PC diagnosis in G84E carriers and non-carriers (59.7 ±8.01 vs. 60.0 ±6.0, p = 0.93). In contrast, Karlsson *et al.* observed in a Swedish population a younger
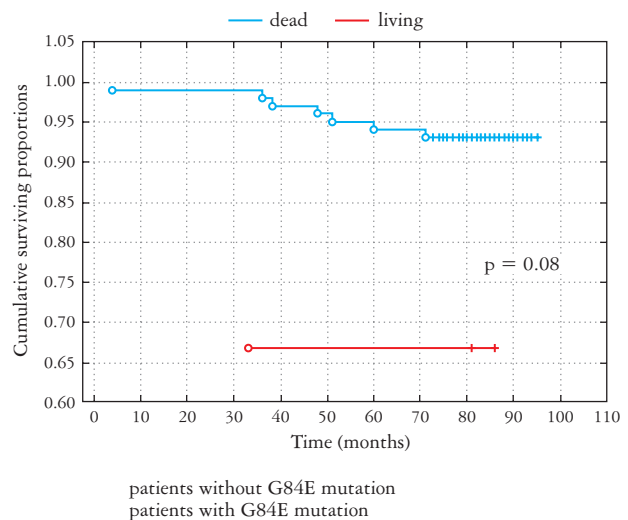
Fig. 2. The Kaplan-Meier probability curves for overall survival from diagnosis of PC for patients with and without HOXB13 G84E mutation. The red line represents the survival for the G84E carriers and the blue line the survival of the men without G84E mutation

mean age at PC diagnosis in G84E carriers than in non-carriers: 62.1 vs. 65.0, respectively; in a Stockholm population: 63.3 vs. 65.9, respectively) [29]. Similarly, Ewing *et al.* demonstrated the relationship between G84E presence and PC development at a younger age (≤ 55 years of age) and with familial aggregation of this cancer (≥ 2 PC relatives). The mutation was present in 2.2% of men with PC detected before 55 years of age and in 0.8% diagnosed after 55 years of age [20]. Thus, further analysis of the age

at PC onset of G84E carriers from the Polish population is needed to elucidate this problem.

We evaluated the relationship between *HOXB13* G84E mutation and five-year survival after PC diagnosis. The differences between the G84E carriers' or non-carriers' survival were not observed by Kluźniak *et al.*; however, they attributed it to the small number of persons in the analysed groups [27]. In the study by Kote-Jarai *et al.*, *HOXB13* G84E presence also was not related to overall survival [19]. In our study, although the study group consisted of only three carriers, a tendency for shorter survival of PC patients with G84E mutation than patients without this mutation could be observed (p = 0.08). Two of three (66%) mutation carriers and 93% of mutation non-carriers survived for five years after prostate cancer diagnosis.

In our study, no statistically significant differences in negative prognostic factors, such as PSA concentration > 20 ng/ml, high-grade Gleason Score (GS ≥ 8), or advanced TNM stage tumours (T3/4), depending on carrier status, were observed. In the study by Kote-Jarai *et al.* there was no association between Gleason Score, prostate-specific antigen level, or tumour-node-metastasis (TNM) stage, and G84E carrier status [19]. However, Storebjerg *et al.*, studying a Danish population, showed that *HOXB13* G84E carriers were significantly more likely to have a higher mean PSA level and pathological Gleason score ≥ 7 at the time at PC diagnosis than non-carriers (respectively, 19.9 vs. 13.6, p = 0.03 and 83.3 vs. 60.9, p = 0.03). The authors indicated that G84E mutation carriers were more likely to develop aggressive PC compared to non-carriers [32].

Conclusions

The results of our study confirm the results obtained by other researchers that *HOXB13* G84E mutation is associated with increased prostate cancer risk. Carriers of this rare mutation are more likely to have a family history of prostate cancer compared with homozygotes for the wild-type allele. Additionally, G84E mutation presence may be associated with older age at PC onset (> 60 years of age) and with shorter survival of mutation carriers. No significant differences in mutation frequency were detected according to selected clinical characteristics of prostate cancer patients.

The authors declare no conflict of interest.

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