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An inherited *NBN* mutation is associated with poor prognosis prostate cancer

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Background: To establish the contribution of eight founder alleles in three DNA damage repair genes (*BRCA1*, *CHEK2* and *NBS1*) to prostate cancer in Poland, and to measure the impact of these variants on survival among patients.

Methods: Three thousand seven hundred fifty men with prostate cancer and 3956 cancer-free controls were genotyped for three founder alleles in *BRCA1* (*5382insC*, *4153delA*, *C61G*), four alleles in *CHEK2* (*1100delC*, *IVS2+1G>A*, *del5395*, *I157T*), and one allele in *NBS1 (657del5)*.

Results: The *NBS1* mutation was detected in 53 of 3750 unselected cases compared with 23 of 3956 (0.6%) controls (odds ratio (OR) = 2.5; $P = 0.0003$). A *CHEK2* mutation was seen in 383 (10.2%) unselected cases and in 228 (5.8%) controls (OR = 1.9; $P < 0.0001$). Mutation of *BRCA1* (three mutations combined) was not associated with the risk of prostate cancer (OR = 0.9; $P = 0.8$). In a subgroup analysis, the *4153delA* mutation was associated with early-onset (age $\leqslant$60 years) prostate cancer (OR = 20.3, $P = 0.004$). The mean follow-up was 54 months. Mortality was significantly worse for carriers of a *NBS1* mutation than for non-carriers (HR = 1.85; $P = 0.008$). The 5-year survival for men with an *NBS1* mutation was 49%, compared with 72% for mutation-negative cases.

Conclusion: A mutation in *NBS1* predisposes to aggressive prostate cancer. These data are relevant to the prospect of adapting personalised medicine to prostate cancer prevention and treatment.

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The number of known genes for which mutations clearly predispose to prostate cancer is small, and include *BRCA2*, *BRCA1, CHEK2*, *NBS1* and *HOXB13* (Struewing *et al*, 1997; Thorlacius *et al*, 1997; Dong *et al*, 2003; Edwards *et al*, 2003; Seppälä *et al*, 2003; Cybulski *et al*, 2004; Kirchhoff *et al*, 2004; Kote-Jarai *et al*, 2011; Ewing *et al*, 2012; Leongamornlert *et al*, 2012). Four of these, *BRCA2*, *BRCA1, CHEK2*, *NBS1* (also known as Nibrin; NBN) are involved in the DNA damage response pathway (Futaki and Lui, 2001). In Poland, we have identified eight founder alleles in three DNA damage repair genes that predispose to breast cancer (Górski *et al*, 2005; Cybulski *et al*, 2011). Three founder alleles are in *BRCA1 (5382insC, 4153delA, C61G)*, four are in *CHEK2 (1100delC, IVS2+1G>A, del5395, I157T)* and one variant allele *(657del5)* is in *NBS1*. To establish the contribution of eight founder alleles in three DNA damage repair genes *(BRCA1, CHEK2 and NBS1)* to prostate cancer in Poland, and to measure the impact of these variants on survival, we genotyped 3750 men with prostate cancer and 3956 controls.

MATERIALS AND METHODS

Patients. We studied men with prostate cancer who were diagnosed between 1999 and 2012 in 14 centres situated throughout Poland. This study was initiated in Szczecin in 1999 and was extended to include Białystok, Olsztyn in 2002 and Opole in 2003. Other centres began recruiting between 2005 and 2008 (Koszalin, Gdansk, Lublin, Łodź, Warszawa, Wrocław, Poznan, Rzeszów, Bydgoszcz, Zabrze). All men with prostate cancer were invited to participate. Study subjects were asked to participate at the time of diagnosis or during an outpatient visit to an oncology clinic and were unselected for age or family history. Four thousand five hundred thirty-one men were invited and of these, 3915 (86.4%) participated. All patients provided a blood sample within 6 months of diagnosis. The mean age of diagnosis was 68.8 years (range 41–96 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 ages of diagnosis were recorded. A family history of cancers in relatives was available for 3586 (92%) subjects. Four hundred sixteen men reported at least one first- or second-degree relative with prostate cancer (familial cases). In addition, information was recorded on PSA level at time of diagnosis, grade (Gleason score) and stage. The study was approved by the Ethics Committee of the Pomeranian Medical University in Szczecin, Poland.

Genotyping. DNA was isolated from 5 to 10 ml of peripheral blood. DNA was successfully isolated from 3853 (98.4%) of 3915 cases. Eight founder mutations in *BRCA1, CHEK2* and *NBS1* were genotyped as described previously (Cybulski *et al*, 2004, 2006; Górski *et al*, 2005). In brief, *BRCA1* mutations, *4153delA* and *5382insC* , were detected using allele-specific oligonucleotide PCR, and C61G was detected using restriction fragment length polymorphism PCR. The *CHEK2 del5395* mutation was detected by a multiplex PCR reaction. The *IVS2+1G>A* and *I157T* variants in *CHEK2* were detected using restriction fragment length polymorphism PCR analysis, and the *1100delC* mutation was analysed using allele-specific oligonucleotide PCR. *NBS1* mutation was tested using allele-specific oligonucleotide PCR. All eight mutations were successfully analysed in 3750 of 3853 cases (97%) including 412 familial prostate cancer cases.

Controls. The control group included 3956 cancer-free men age 23–90 years (mean age, 61.2 years). The purpose of the control group was to estimate with accuracy the frequency of founder alleles of *BRCA1*, *CHEK2* and *NBS1* in the underlying Polish population. These controls were derived from four sources. The first series consisted of consisted of 603 unselected men (age range, 30–90 years; mean age, 64.2 years) selected at random from the computerised patient lists of five large family practices located in the region of Szczecin. These were invited to participate by mail and participated in 2003 and 2004. The second subgroup consisted of 1008 men from the region of Szczecin (age range, 23–87 years; mean age, 61.6 years). These men were part of a population-based study of the 1.5 million residents of West Pomerania designed to identify familial cancer clusters and were interviewed in 2007. Men with any cancer diagnosed in a first-degree relative were excluded from this control group. The third series consisted of 1301 unselected men at age above 45 (age range, 45–90 years; mean age, 61.9 years) with PSA level below 4.0 ng l^{-1}. These men were selected randomly from a database of a population-based study of the 1.5 million residents of West Pomerania and provided blood sample between 2010 and 2012. Men with PSA above 4.0 ng l^{-1} and men with a positive family history of prostate cancer were excluded from this group. The fourth series included 1044 Polish men (age range, 55–66 years; mean age, 60.1 years), who participated in population colonoscopy screening programme for colorectal cancer between 2005 and 2010, and provided blood samples for DNA analysis (771 men were from Szczecin, 189 from Białystok and 84 from Łodz). The allele frequencies for all variants in our control group were not dependent on age, and the prevalence estimates of mutations in all genes were similar in younger and in older controls. The frequency of I157T in our controls (4.7%) is similar to that reported by Brennan *et al* (2007) in a non-overlapping series of 790 controls from Poland (5.6%). The frequency of 1100delC in our controls and in controls genotyped by Brennan *et al* (2007) is 0.2%. The frequency of *NBS1* in our controls and in 6984 (non-overlapping) controls genotyped by Chrzanowska *et al* (2006) is 0.6%.

Statistical analysis. The prevalences of all alleles in cases and controls were compared. Odds ratios (ORs) were generated from two-by-two tables and statistical significance was assessed with the Fisher exact test or the χ^2 test where appropriate. The ORs were used as estimates of relative risk. For the survival analysis, the patients were followed from the date of biopsy until death or March 2012. The vital status and the date of death were requested from the Polish Ministry of the Interior and Administration in March 2012, and were obtained in April 2012. These data were available for 3487 (93%) of 3750 men with prostate cancer.

The mean follow-up (overall, 54.4 months) was 67.5 months for *BRCA1* carriers ($P=0.2$), 53.6 months for *NBS1* carriers ($P=0.9$), 57.1 months for *CHEK2* carriers ($P=0.1$), compared with 54.0 months in non-carriers. Mean follow-up was compared using *t*-test.

Kaplan–Meier survival curves were constructed for carriers of mutations in either of the three genes and for non-carriers. Comparison of survival curves was performed by log-rank test. For a subset of 1804 patients (including 37 *NBS1* mutation carriers and 1767 non-carriers) survival data and detailed tumour characteristics were available (PSA level at diagnosis, tumour stage and Gleason score). A multivariable Cox regression analysis was performed, including age of diagnosis, year of diagnosis, PSA level at diagnosis, Gleason score and stage (T1–4) as covariates.

RESULTS

A mutation in one of the three DNA damage repair genes was seen in 443 of 3750 (11.8%) patients with prostate cancer and in 190 of 2912 (6.5%) controls (Table 1). Strong associations were seen for both *CHEK2* and *NBS1*. The single *NBS1* mutation (*657del5*) was detected in 53 of 3750 unselected cases (OR = 2.5; $P=0.0003$) and in 10 of 412 familial cases (OR = 4.3; $P=0.0001$) compared with

Table 1. Association of variant alleles in BRCA1, NBS1 and CHEK2 with prostate cancer risk

	Controls ($n=3956$) No. (%)	Unselected cases ($n=3750$) No. (%)	OR	95% CI	*P*-value	Familial cases ($n=412$) No. (%)	OR	95% CI	*P*-value
Any BRCA1 mutation	*17 (0.4%)*	*14 (0.4%)*	*0.9*	*0.4–1.8*	*0.8*	*4 (1.0%)*	*2.3*	*0.8–6.8*	*0.3*
5382insC	13 (0.3%)	6 (0.2%)	0.5	0.2–1.3	0.2	1 (0.2%)	0.7	0.1–5.7	0.8
C61G	3 (0.08%)	3 (0.08%)	1.1	0.2–5.2	0.9	2 (0.5%)	6.4	1.1–38.6	0.1
4153delA	1 (0.03%)	5 (0.13%)	5.3	0.6–45.2	0.2	1 (0.2%)	9.6	0.6–154	0.5
NBS1 mutation									
657del5	23 (0.6%)	53 (1.4%)	2.5	1.5–4.0	0.0003	10 (2.4%)	4.3	2.0–9.0	0.0001
Any *CHEK2* mutation	228 (5.8%)	383 (10.2%)	1.9	1.6–2.2	<0.0001	59 (14.3%)	2.7	2.0–3.7	<0.0001
Any CHEK2 truncating mutation	*43 (1.1%)*	*84 (2.2%)*	*2.1*	*1.4–3.0*	*0.0001*	*16 (3.9%)*	*3.7*	*2.1–6.6*	*<0.0001*
1100delC	7 (0.2%)	21 (0.6%)	3.2	1.4–7.5	0.009	4 (1.0%)	5.5	1.6–19.0	0.01
IVS2+1G>A	21 (0.5%)	28 (0.7%)	1.4	0.8–2.5	0.3	7 (1.7%)	3.2	1.4–7.7	0.01
del5395	15 (0.4%)	35 (0.9%)	2.5	1.3–4.5	0.004	5 (1.2%)	3.2	1.2–8.9	0.04
CHEK2 I157T missense mutation	186 (4.7%)	303 (8.1%)	1.8	1.5–2.2	<0.0001	43 (10.4%)	2.4	1.7–3.3	<0.0001

Abbreviations: CI = confidence interval; HR = hazard ratio. Familial cases – prostate cancers in two or more first- or second-degree relatives. One control carried two mutations (I157T and 1100delC). Eleven cases carried two mutations (four cases had I157T and a *CHEK2* truncating mutation, four carried I157T and *NBS1* mutation, two carried I157T and a *BRCA1* mutation, and one carried a *CHEK2* truncating mutation and a *BRCA1* mutation).

23 of 3956 (0.6%) controls. A *CHEK2* mutation was seen in 383 (10.2%) unselected cases of prostate cancer (OR = 1.9; $P<0.0001$), in 59 (14.3%) familial cases (OR = 2.7; $P<0.0001$) and in 228 (5.8%) controls. A *BRCA1* mutation (three *BRCA1* mutations combined) was not associated with the risk of prostate cancer (OR = 0.9; $P=0.8$). Although not statistically significant ($P=0.2$), the *4153delA* mutation was associated with OR of 5.3 for prostate cancer, but the other two mutations of *BRCA1* were not associated with an increase in the risk of prostate cancer (OR = 0.5 for the *5382insC* mutation; OR = 1.1 for the *C61G* mutation).

We also investigated the incidence of the mutations by age, dividing cases into two groups: men diagnosed at age of 60 and below, and men diagnosed at age above 60 years (Table 2). For all genes, the mutation frequencies and the ORs were higher for early-onset cases than for cases diagnosed above age of 60 years. The ORs for early-onset prostate cancer were 3.1 ($P=0.003$) for *NBS1* mutation, 2.3 ($P<0.0001$) for a *CHEK2* mutation and 1.9 ($P=0.9$) for a *BRCA1* mutation. For cases diagnosed above age of 60 years the ORs were 2.4 ($P=0.0009$) for *NBS1* mutation, 1.8 ($P<0.0001$) for a *CHEK2* mutation and 0.7 ($P=0.6$) for *BRCA1* mutation. Among younger cases, the OR for BRCA1 (all three variants combined) was 1.9 ($P=0.9$) and for the *4153delA* mutation alone was 20.3 ($P=0.004$).

The characteristics of the prostate cancer cases in the 443 carriers and 3307 non-carriers are presented in Table 3. There were few significant differences between subgroups; men with a *CHEK2* mutation were diagnosed with prostate cancer on average 1.4 years younger than non-carriers (67.5 *vs* 68.9; $P=0.003$). Prostate cancers of advanced stage (T4) were more common in carriers of a *NBS1* mutation than in non-carriers (19.5% *vs* 7.7%; $P=0.01$).

In addition, tumours of Gleason grade 8–10 were more frequent in men with a *NBS1* mutation than in non-carriers (28.4% *vs* 19.1%), but this difference was not statistically significant ($P=0.1$).

Data on survival was available for 3487 men with prostate cancer (Table 4). There were five deaths (38.5%) recorded in 13 carriers of a *BRCA1* mutation, 19 deaths (36.5%) in 52 carriers of a *NBS1* mutation, 87 deaths (25.6%) in 340 men with a *CHEK2* mutation and 755 deaths (24.5%) in 3082 non-carriers. Kaplan–Meier survival curves for mutation carriers and non-carriers are shown in Figure 1. The mortality experience was significantly worse for carriers of a *NBS1* mutation, compared with non-carriers (HR = 1.85; $P=0.008$). The poor relative survival for carriers of an *NBS1* mutation was particularly apparent in the first 5 years after diagnosis (HR = 2.08; $P=0.002$). The 5-year survival for carriers of a *NBS1* mutation was 49%, compared with 72% for non-carrier controls. After adjusting for age, year of diagnosis, PSA, stage and grade, the HR for mortality associated with a *NBS1* mutation was 1.86 (95% CI, 1.05–3.32; $P=0.04$). Of the 52 carriers of a *NBS1* mutation, 19 (36.5%) have died, on average 24.3 months after diagnosis. The characteristics of the patients and the corresponding tumours for the 19 fatal cases among NBS1 mutation carriers is presented in Table 5.

The survival experience of carriers of a *BRCA1* mutation was also relatively poor, but this subgroup was small ($n=13$ *BRCA1* mutation carriers) and the difference was not statistically significant (HR = 1.48; $P=0.38$). Survival in men with a *CHEK2* mutation was similar to that of non-carriers (HR = 0.99 and $P=0.95$).

DISCUSSION

The most noteworthy observation is the remarkably poor short-term survival of men with prostate cancer and *NBS1* mutation. We have confirmed our earlier work that describes *NBS1* as a prostate cancer susceptibility gene (Cybulski *et al*, 2004), and we have extended our findings by documenting the aggressive nature of the associated tumours. The *NBS1 657del5* founder allele is present in ~1 in 170 individuals in Poland and is associated with a three-fold increased risk of prostate cancer. Cancers in carriers of the *NBS1 657del5* founder mutation are typically aggressive; ~30% were of Gleason grade 8 or above and approximately one-half of the patients with this allele died within 5 years of diagnosis. Compared with men with no mutation, the relative survival rate at 5 years was only 48%. The aggressive behaviour of these cancers was not entirely attributable to the grade, after adjustment for age, grade, stage and PSA, the *NBS1*-associated cancers had a relatively poor survival (HR = 1.86; 95% CI, 1.05–3.32; $P=0.04$). In Poland, ~1.4% of prostate cancers are attributable to a mutation of *NBS1* and 5.5% are due to *CHEK2* mutations. However, in terms of prognosis, the cancers in carriers of *CHEK2* mutations are not distinguishable from cancers in the population at large. To our knowledge, this is the first study to describe the clinical

Table 2. Association of variant alleles in BRCA1, NBS1 and CHEK2 with prostate cancer risk, by age

	Controls ($n=3956$) No. (%)	Cases diagnosed at age $\leqslant 60$ years ($n=619$) No. (%)	OR	95% CI	*P*-value	Cases diagnosed at age >60 years ($n=3131$) No. (%)	OR	95% CI	*P*-value
Any BRCA1 *mutation*	*17 (0.4%)*	*5 (0.8%)*	*1.9*	*0.7–5.1*	*0.9*	*9 (0.3%)*	*0.7*	*0.3–1.5*	*0.6*
5382insC	13 (0.3%)	1 (0.2%)	0.5	0.1–3.8	0.8	5 (0.2%)	0.5	0.2–1.4	0.2
C61G	3 (0.08%)	1 (0.2%)	2.1	0.2–20.5	1.0	2 (0.06%)	0.8	0.1–5.0	0.8
4153delA	1 (0.03%)	3 (0.5%)	20.3	2.0–185.6	0.004	2 (0.06%)	2.5	0.2–27.9	0.8
NBS1 mutation									
657del5	23 (0.6%)	11 (1.8%)	3.1	1.5–6.4	0.003	43 (1.4%)	2.4	1.4–4.0	0.0009
Any CHEK2 mutation	228 (5.8%)	77 (12.4%)	2.3	1.8–3.1	<0.0001	306 (9.8%)	1.8	1.5–2.1	<0.0001
Any CHEK2 *truncating mutation*	*43 (1.1%)*	*16 (2.6%)*	*2.4*	*1.4–4.3*	*0.004*	*68 (2.2%)*	*2.0*	*1.4–3.0*	*0.0004*
1100delC	7 (0.2%)	4 (0.6%)	3.7	1.1–12.6	0.08	17 (0.5%)	3.1	1.2–7.4	0.02
IVS2+1G>A	21 (0.5%)	4 (0.6%)	1.2	0.4–3.6	0.9	24 (0.8%)	1.4	0.8–2.6	0.3
del5395	15 (0.4%)	8 (1.3%)	3.4	1.5–8.2	0.007	27 (0.9%)	2.3	1.2–4.3	0.01
CHEK2 I157T missense mutation	186 (4.7%)	62 (10.0%)	2.3	1.7–3.0	<0.0001	241 (7.7%)	1.7	1.4–2.1	<0.0001

Abbreviations: CI = confidence interval; HR = hazard ratio. ORs and *P*-values are calculated with respect to controls as reference group.

Table 3. Clinical characteristics of prostate cancers in carries of variant alleles in BRCA1, NBS1, CHEK2 and in non-carriers

	***BRCA1* mutation carriers ($n=14$)**	***P*-value**	***NBS1*mutation carriers ($n=53$)**	***P*-value**	***CHEK2* mutation carriers ($n=383$)**	***P*-value**	**Mutation-negative cases ($n=3307$)**
Age of diagnosis							
Mean	68.3	0.8	67.3	0.2	67.5	0.003	68.9
PSA level at diagnosis							
Median	14.5	0.6	10.7	0.8	10.9	0.3	11.2
$\leqslant 4.0$	10.0% (1/10)	0.9	2.8% (1/36)	0.9	5.3% (13/247)	0.7	4.5% (112/2474)
4.1–10	30.0% (3/10)	0.7	47.2% (17/36)	0.5	40.5% (100/247)	1.0	40.4% (999/2474)
10.1–20.0	20.0% (2/10)	1.0	25.0% (9/36)	0.8	26.3% (65/247)	0.7	25.0% (619/2474)
>20.0	40.0% (4/10)	0.7	25.0% (9/36)	0.6	27.9% (69/247)	0.5	30.1% (744/2474)
Gleason score							
<7	36.4% (4/11)	0.5	43.6% (20/46)	0.3	55.2% (142/257)	0.3	51.9% (1341/2584)
7	45.4% (5/11)	0.4	28.2% (13/46)	1.0	26.1% (67/257)	0.4	29.0% (749/2584)
>7	18.2% (2/11)	0.8	28.2% (13/46)	0.1	18.7% (48/257)	0.9	19.1% (494/2584)
Stage							
T3	30% (3/10)	0.4	14.6% (6/41)	0.8	18.6% (40/215)	1.0	17.8% (362/2029)
T4	20% (2/10)	0.2	19.5% (8/41)	0.01	6.0% (13/215)	1.0	7.7% (156/2029)
Family history of prostate cancer							
Positive	30.8% (4/13)	0.07	20.0% (10/50)	0.07	16.9% (59/332)	0.001	11.0% (341/3095)

P-values for mutation carriers are calculated with respect to non-carriers as reference group.

characteristics and survival of men with prostate cancer and a mutation in *NBS1* and *CHEK2*.

There is no organised prostate screening programme in Poland and the majority of the patients in this study presented because of symptoms or because of an abnormal digital rectal examination. Our results are of interest in considering whether or not prostate cancer screening is warranted in Poland, and if so, if a screening programme should be universal or personalised (i.e., targeted to those at high risk). Personalised screening might incorporate two phases – the first phase would screen men for the five susceptibility alleles described here. Men with a mutation in one of the two genes would then be a candidate for PSA-based prostate cancer screening. In particularly men with an *NBS1* mutation might be screened aggressively, perhaps including a random biopsy. In our study, the earliest age of onset among men with a *NBS1* mutation was 50 years and among men with a *CHEK2* mutation was 45 years. However, the principal limitation of this personalised model is that only 12% of new cases of prostate cancer in Poland occur in men with one of these mutations and therefore the potential to reduce the overall cancer burden is limited. Also, the benefit or

Table 4. Survival of men with a mutation in BRCA1, NBS1 and CHEK2 and in non-carriers

	BRCA1 mutation (*n* = 13)	*NBS1* mutation (*n* = 52)	*CHEK2* mutation (*n* = 340)	Mutation-negative cases (*n* = 3082)
Mean follow-up (months)	67.5	53.6	57.1	54.0
Proportion of deceased (%)	38.5	36.5	25.6	24.5
Median survival (months)	51	57	122	121
5-Year survival (%)	46	49	71	72
10-Year survival (%)	46	39	56	52
HR	1.48	1.85	0.99	1.0[a]
95% CI	0.51–4.30	1.17–2.91	0.80–1.24	—
P-value	0.38	0.008	0.95	—

Abbreviations: CI = confidence interval; HR = hazard ratio.
HR, 95% CI and *P*-values are calculated by log-rank test.
[a]Reference value.

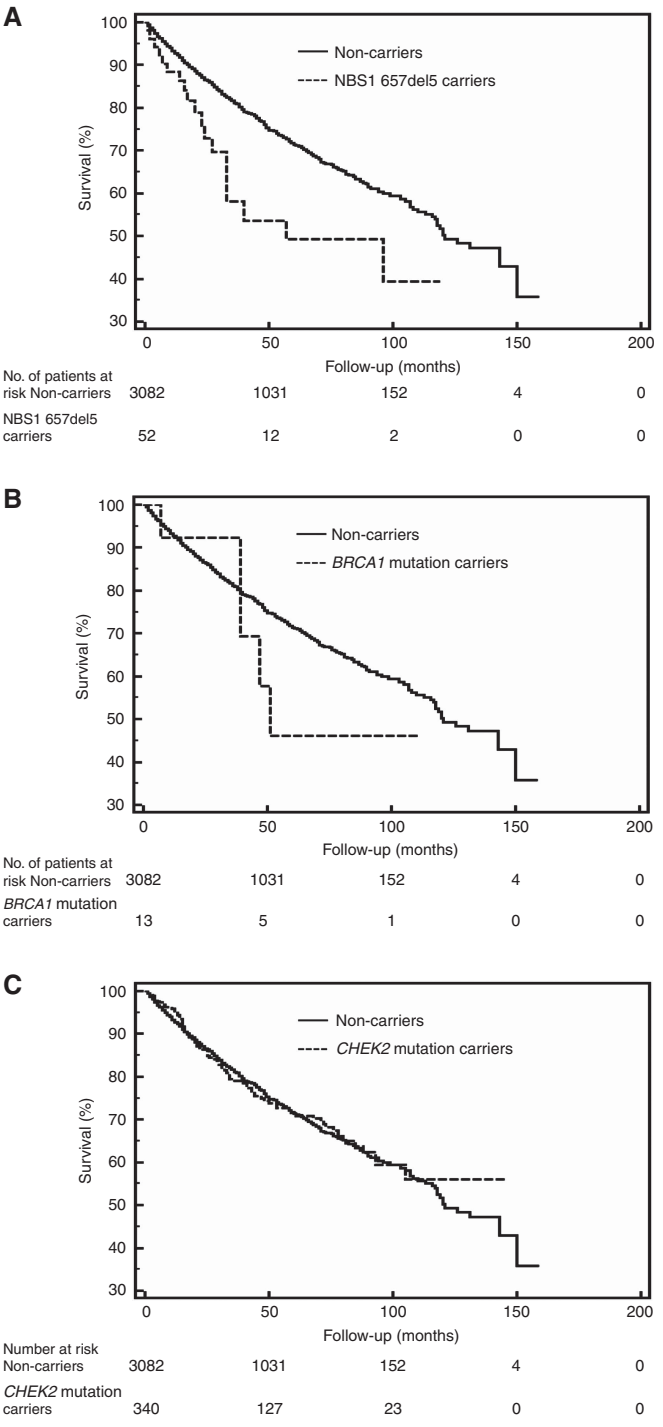

Figure 1. Kaplan–Meier curves of prostate cancer patients with: (**A**) mutation in *NSB1* (*n* = 52); (**B**) mutation in *BRCA1* (*n* = 13); (**C**) mutation in *CHEK2* (*n* = 340), compared with prostate cancer patients with no mutation (non-carriers; *n* = 3082).

prostate screening on reducing mortality in average risk men using the conventional PSA test has not been proven and has not been evaluated in men with predisposing mutations (Djulbegovic *et al*, 2010, 2012; Schröder *et al*, 2012). Only one study called IMPACT (Identification of Men with a genetic predisposition to ProstAte Cancer: Targeted screening in *BRCA1/2* mutation carriers and controls; http://www.impact-study.co.uk) investigated prostate cancer screening targeted at men with a known genetic predisposition to the disease. Preliminary analysis of the data from the IMPACT study supports the rationale for continued PSA screening in such men (Mitra *et al*, 2011). The IMPACT study only referred to germline mutations in *BRCA1* and *BRCA2*, and it is not know if men with *CHEK2* and *NBS1* mutations should be thus screened, but are good candidates for study.

NBS1, also known as Nibrin (NBN), is the gene for Nijmegen breakage syndrome (NBS), a rare autosomal recessive disorder that is characterised by immunodeficiency, chromosomal instability and sensitivity to ionising radiation (Varon *et al*, 1998). The *657del5* mutation is responsible for 90% of all reported cases of NBS to date (Varon *et al*, 2000). On the basis of the geographic distribution of reported clinical cases of Nijmegen syndrome, the distribution of *657del5* allele of *NBS1* is not worldwide, and this allele is most common in Slavic populations of Eastern Europe. Other truncating mutations of *NBS1* (*698del4* of English origin, *835del4* of Italian origin, *842insT* of Mexican origin, *1142delC* of Canadian origin, and *Q326X* of Dutch origin) were detected in 10% of NBS patients (Varon *et al*, 1998), but their geographic extend and their role in prostate cancer susceptibility has not been established. Only one previous study explored the association between *NBS1 657del5* and prostate cancer risk. In that study, the *657del5* allele was seen in 7 (0.23%) of 3037 men with prostate cancer and in none of 990 unaffected controls in the United States (Hebbring *et al*, 2006). The clinical characteristics of the mutation-positive cases is not described.

The *NBS1* founder allele is predicted to result in a truncated protein of 219 of 754 amino acids (p26) (Maser *et al*, 2001). However, the *657del5* allele also creates an aberrant translation initiation site, which generates a partially functional variant of the *NBS1* protein (p70). Null mutations in MRE11 and RAD50, which encode binding partners of *NBS1*, are lethal in vertebrates, and mouse *Nbs1*-null mutants are inviable (Maser *et al*, 2001). Therefore, it is likely that truly null mutations of *NBS1* (in homozygous state) do not cause Nijmegen syndrome, but only those mutations that encode partially active Nibrin (such as the *657del5* mutation) are pathogenic for the syndrome. However, null mutations of *NBS1* in heterozygous state are not lethal, and may well predispose to cancer. It is therefore possible that cancer-associated mutations of *NBS1* are different from NBS-related mutations. *NBS1* needs to resequenced in cancer patients to describe full spectrum of cancer predisposing mutations in

Table 5. The characteristics of the patients and the corresponding tumours for the 19 fatal cases with the 657del5 mutation in NBS1

***NBS1* mutation-positive lethal prostate cancer cases ($n=19$)**		
Age (years)	Mean age (range)	69.1 (52–86)
Age group	⩽60	21.1% (4/19)
PSA	61–70	31.6% (6/19)
	>70	47.3% (9/19)
	Median (range)	20.5 (6–190)
	⩽4.0	0.0% (0/12)
	4.1–10.0	33.3% (4/12)
	10.1–20.0	16.7% (2/12)
	>20.0	50.0% (6/12)
Gleason score	<7	40.0% (6/15)
	7	26.7% (4/15)
	>7	33.3% (5/15)
Stage	T3	23.1% (3/13)
	T4	38.4% (5/13)
Family history of prostate cancer	Positive	11.8% (2/17)

Abbreviation: PSA = prostate-specific antigen.

different ethnic groups (regardless of geographic distribution of NBS syndrome). Of note, recently, 94 unrelated familial prostate cancer cases from the United States were screened by next-generation sequencing (whole exome-sequencing). A novel truncating mutation of *NBN*, *2117 C>G* mutation that results in a premature stop at codon 706 (*S706X*) was detected in one family, and the mutation partially cosegregated with prostate cancer in this family (Zuhlke *et al*, 2012).

The situation of *NBS1* carriers is similar to that of men with prostate cancer with a mutation in *BRCA2* (in Poland, there are no known founder mutations in *BRCA2* and the gene was not part of the current analysis). Men with prostate cancer and a *BRCA2* mutation have a poor prognosis despite conventional therapy (prostatectomy, hormonal therapy, radiation therapy), and most *BRCA2* carriers with prostate cancer will succumb to their disease (Sigurdsson *et al*, 1997; Narod *et al*, 2008; Edwards *et al*, 2010; Thorne *et al*, 2011). Both *NBS1* and *BRCA2* genes act in DNA damage repair signalling pathway, and mutations in the two genes (in homozygous state) cause inherited recessive clinical syndromes, such as NBS (*NBS1* mutation), Fanconi anaemia (*BRCA2* mutation), which are characterised by spontaneous chromosomal instability, immunodeficiency and a predisposition to cancer (Digweed, 1993; Varon *et al*, 1998; Howlett *et al*, 2002). It will be of interest to determine whether mutations of other genes for the chromosomal instability syndromes (*BLM* gene for Bloom syndrome, FA genes for Fanconi anaemia, *ATM* gene for ataxia telangiectasia) also predispose to aggressive prostate cancer.

It is also important to determine whether therapy beyond the conventional therapy is valuable for men with prostate cancer and a *NBS1* mutation (or a *BRCA2* mutation). Similarly to *BRCA2*, *NBS1* appears to act as a classical tumour-suppressor gene (Willems *et al*, 2008). Biallelic *NBS1* inactivation was observed in most tumours in *657del5* carriers and the cancers that develop in the prostates of carriers are functionally homozygous for the mutation (Cybulski *et al*, 2004). The product of the *NBS1* gene is an integral component of the Mre11/Rad50/NBS1 nuclease complex, which has a role in the initial processing of double-strand DNA breaks before repair by homologous recombination (Petrini, 1999; Lee and Paull, 2004, 2005). If double-strand DNA breaks are not recognised, then the repair is impaired. Therefore, men with prostate cancer and *NBS1* mutation (or a *BRCA2* mutation) might benefit from treatment with cisplatin and PARP1 inhibitors.

We saw little effect of a *BRCA1* mutation (all three mutations combined) on prostate cancer risk (OR = 0.9; $P=0.8$), whereas several previous studies suggested an effect (Ford *et al*, 1994; Struewing *et al*, 1997; Warner *et al*, 1999; Thompson and Easton, 2002a; Giusti *et al*, 2003; Leongamornlert *et al*, 2012). However, we observed excess of *BRCA1* mutations in men with early-onset prostate cancer (⩽60 years), but this did not achieve statistical significance (OR = 1.9; $P=0.9$) possibly because of small numbers. Our data are in line with the results of the Breast Cancer Linkage Consortium, which reported an increase in prostate cancer risk in carriers of a *BRCA1* mutation aged <65 years with a RR of 1.82 (95% CI 1.01–3.29, $P=0.05$), but no risk increase was seen for men ⩾65 years (Thompson and Easton, 2002a). It is also interesting that in our study, only the *4153delA* mutation was associated with increased risk of unselected prostate cancer (OR = 5.3, $P=0.2$) and of early-onset (⩽60 years) prostate cancer (OR = 20.3; $P=0.004$), but the other two *BRCA1* mutations (*5382insC* and *C61G*) were not. This is a subgroup analysis and may be due to chance, but it is also possible that there is a genotype–phenotype effect in the *BRCA1* gene, wherein only some mutations (such as *4153delA*) are pathogenic for prostate cancer. Genotype–phenotype correlations have been suggested for breast and ovarian cancer risk in *BRCA1* carriers (Gayther *et al*, 1995; Holt *et al*, 1996; Neuhausen *et al*, 1996; Thompson and Easton, 2002b; Rennert *et al*, 2005; Gronwald *et al*, 2006). It is also possible that the risk of prostate cancer may vary by the type and/or location of the *BRCA1* mutation, but further studies are needed.

In conclusion, our results provide compelling evidence that a founder mutation in *NBN* predisposes to aggressive prostate cancers. The data presented here raise important questions about the prospect of adapting personalised medicine to prostate cancer prevention.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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APPENDIX

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