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 ≤ 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 BRCA1, BRCA2, CHEK2, NBS1 and HOXB13 (Struwing et al, 1997; Thorlacius et al, 1997; Dong et al, 2003; Edwards et al, 2003; Seppälä et al, 2003; Cylburski 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; Cylburski 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, Gdańsk, Lublin, Łódź, 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 (Cylburski 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 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 Łódź). 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 \(z^2\) 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 (657delS) 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
23 of 3956 (0.6%) controls. A CHEK2 mutation was seen in 383 (10.2%) unselected cases of prostate cancer (OR = 1.9; 95% CI: 0.95–3.92, P = 0.03) and in 59 (14.3%) familial cases (OR = 2.7; 95% CI: 1.3–5.5, 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 are 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; 95% CI: 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 associations of CHEK2 and NBS1 with prostate cancer risk.

| Variant Alleles | Controls (n = 3956) | Unselected cases (n = 3750) | OR | 95% CI | P-value | Familial cases (n = 412) | 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 | 0.5–2.5 | 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 truncating 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 |
| 1100delC | 43 (1.1%) | 84 (2.2%) | 2.1 | 1.7–3.0 | 0.0001 | 16 (3.9%) | 3.7 | 2.1–6.6 | <0.0001 |
| IVS2 + 1G > A | 7 (0.2%) | 21 (0.5%) | 3.2 | 1.4–7.5 | 0.009 | 4 (1.0%) | 5.5 | 1.6–19.0 | 0.01 |
| del5395 | 15 (0.4%) | 35 (0.9%) | 1.4 | 0.8–2.9 | 0.3 | 4 (1.0%) | 3.2 | 1.4–7.7 | 0.01 |
| 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 (157T and 1100delC). Eleven cases carried two mutations (four cases had 157T and a CHEK2 truncating mutation, four carried 157T and NBS1 mutation, two carried 157T and a BRCA1 mutation, and one carried a CHEK2 truncating mutation and a BRCA1 mutation).
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 2. Association of variant alleles in BRCA1, NBS1 and CHEK2 with prostate cancer risk, by age |
|---------------------------------------------------------------|
| **Controls (n = 3956)** | **Cases diagnosed at age < 60 years (n = 619)** | **OR** | **95% CI** | **P-value** | **Cases diagnosed at age > 60 years (n = 3131)** | **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 (0.0%)                        | 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 |
| 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          | 186 (4.9%)                      | 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 carriers 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 |
| <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.
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.impactsudy.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 known 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 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 (698delC 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., 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 (698delC 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 of 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 be sequenced in cancer patients to describe full spectrum of cancer predisposing mutations in

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

| Gene   | Mutation (n) | Mutations | Mutation-positive cases |
|--------|--------------|-----------|------------------------|
| BRCA1  | mutation (n = 13) | 657del5   | 67.5 53.6 57.1 54.0 |
| NBS1   | mutation (n = 52)  | 657del5   | 38.5 36.5 25.6 24.5 |
| CHEK2  | mutation (n = 340) | 657del5   | 51 57 122 121 |
|        | mutation (n = 3082) | 657del5   | 46 49 71 72 |
|        | mutation (n = 2012) | 657del5   | 100 100 100 100 |

| Survival (%) | 5-Year | 10-Year | HR 95% CI P-value |
|--------------|--------|---------|------------------|
| Non-carriers | 46     | 46      | 1.48 1.85 0.99 1.0 |
| NBS1 657del5 carriers | 49     | 39      | 0.51–4.30 1.17–2.91 0.80–1.24 0.38— |

Abbreviations: CI = confidence interval; HR = hazard ratio. P-values are calculated by log-rank test.

*Reference value.

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**Figure 1.** Kaplan-Meier curves of prostate cancer patients with: (A) mutation in NBS1 (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).
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 in NBN (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 anemia (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 anemia, 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; Struwing 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 some mutations 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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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.
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APPENDIX

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