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# Selected aspects of inherited susceptibility to prostate cancer and tumours of different site of origin

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## Abstract

Epidemiologic research conducted over the last two decades has led us to believe that inherited factors play an important role in the aetiology of prostate cancer, but the genes which underlie the inherited susceptibility are elusive. The most compelling associations to date are with genes involved in DNA damage repair, including *BRCA2*. In Poland we have initiated a programme to identify DNA variants which confer an increased risk of prostate cancer and other cancers. Here we review our recent results. We found that germline mutations in *BRCA1*, *CHEK2* and *NBS1* confer an increased prostate cancer risk in Polish men. We provide evidence that *CHEK2* is a multi-organ cancer susceptibility gene. We show that inherited variation in *RNASEL* and *MSR1* genes do not contribute to prostate cancer development in Poland.

## Introduction

Research conducted over the last two decades has led us to believe that inherited factors play an important role in the aetiology of cancer [1-8]. Prostate cancer is among the leading causes of morbidity and mortality in men. Relatively little is known about the genetic determinants of this disease, but epidemiologic data suggest that dominant susceptibility genes may be responsible for up to 5% of all of cases [9-10]. Through linkage analysis, numerous chromosomal loci have been identified, but no clear prostate susceptibility gene has emerged. Three candidate susceptibility genes have been positionally cloned – *HPC1*, *HPC2/ELAC2* and *MSR1* – but a clear role for any of these genes in hereditary prostate cancer has not been established [11, 12]. There is evidence that rare mutations of genes in the DNA damage signalling pathway and cell cycle control pathway (*BRCA2*, *CHEK2* and *NBS1*) predispose to prostate cancer, but the contribution of these two genes to prostate cancer aetiology is relatively small [13]. Common variants in the genes in these pathways (*CDKN1B*, *CDKN1A*, *ATMATM*, *XRCC1*, *ERCC2*) also have been associated with an increased risk of prostate cancer [14-16]. The DNA damage signalling and cell cycle control pathways play a crucial role in the maintenance of the integrity of the genome in response to DNA damage and has been implicated in the pathogenesis of prostate cancer and of cancers at other sites. This paper reviews a range of studies which have been performed in Polish population with the following objectives:

1) to investigate the association between inherited variation in *RNASEL*, *MSR1*, *NBS1* and *BRCA1* genes and prostate cancer risk in the Polish population;
2) to investigate the role of *CHEK2* mutations in inherited susceptibility to prostate cancer and malignancies of other sites in the Polish population.

# 1. Inherited variation in *RNASEL*, *MSR1*, *NBS1* and *BRCA1* genes and prostate cancer risk in the Polish population

## 1.1. DNA variants in *RNASEL* and *MSR1* genes and susceptibility to prostate cancer

(based on: Cybulski C, Wokołorczyk D, Jakubowska A, Gliniewicz B, Sikorski A, Huzarski T, Debniak T, Narod SA, Lubiński J. DNA variation in *MSR1*, *RNASEL* and *E-cadherin* genes and prostate cancer in Poland. Urol Int 2007; 79: 44-49)

*RNASEL* and *MSR1* were identified through linkage studies of prostate cancer families. Two mutations in *RNASEL* (Met1Ile and Glu265X) were originally described in familial prostate cancer cases from the USA [17]. Other more common variants in *RNASEL* (R462Q and D541E) were found to influence the risk of prostate cancer in men [18]. Germline mutations in the *MSR1* gene (six rare missense variants and R293X truncating mutation) were first shown to segregate with hereditary prostate cancer in the USA. Common polymorphisms in *MSR1* have been associated with increased prostate cancer risk [19]. To date, the roles of *RNASEL* or *MSR1* genes in prostate cancer aetiology have not been investigated in Slavic populations. We investigated if inherited variation in these genes influences prostate cancer risk in Poland.

### Materials and methods

The case group consisted of 737 prostate cancer cases. Of the 737 cases, 506 were from Szczecin and 231 were from other countries (Opole, Bialystok, Olsztyn). Study subjects were unselected for age or family history. Family histories were obtained from each subject. 110 patients had one or more first- or second-degree relatives with prostate cancer (familial cases). The control group consisted of 511 unselected healthy elderly men aged 50 and above, taken from three family doctors practicing in Szczecin. None of the controls had cancer.

The polymorphisms in *MSR1* and *RNASEL* were selected after sequencing of the entire coding region of these genes in 52 and 94 Polish men with familial prostate cancer, respectively. Sequencing revealed only two variants in the *RNASEL* gene (R462Q and D541E) and two variants in the coding sequence of the *MSR1* gene (P275A and R293X). These DNA variants were then genotyped by restriction fragment length polymorphism polymerase chain reaction (RFLP-PCR) using the restriction enzymes AvaI (R462Q variant), MboI (D541E variant), Hpy8I (P275A variant) and MvaI (R293X variant).

### Results

The prevalence of the sequence variants in cases and controls is shown is Table 1. The R462Q and D541E

**Table 1.** Comparison of the frequency of variants in *RNASEL* and *MSR1* genes in 737 patients with prostate cancer and 511 individuals from control group

| Gene | Variant | Genotype | Number of carriers (frequency) | | OR | 95% CI | p |
|---|---|---|---|---|---|---|---|
| | | | cases (n=737) No. (%) | controls (n=511) No. (%) | | | |
| *RNASEL* | 1385G>A (R462Q) | GG | 245 (33.3) | 177 (34.6) | 0.9 | 0.7-1.2 | 0.6 |
| | | GA | 376 (51.0) | 252 (49.3) | 1.1 | 0.9-1.3 | 0.6 |
| | | AA | 116 (15.7) | 82 (16.1) | 1.0 | 0.7-1.3 | 0.9 |
| | 1623T>G (D541E) | TT | 111 (15.1) | 84 (16.4) | 0.9 | 0.7-1.2 | 0.5 |
| | | TG | 372 (50.5) | 259 (50.7) | 1.0 | 0.8-1.2 | 1.0 |
| | | GG | 254 (34.4) | 168 (32.9) | 1.1 | 0.8-1.4 | 0.6 |
| *MSR1* | 945C>G (P275A) | CC | 663 (90.0) | 474 (92.8) | 0.7 | 0.5-1.1 | 0.1 |
| | | CG | 74 (10.0) | 37 (7.2) | 1.4 | 0.9-2.2 | 0.1 |
| | 999C>T (R293X) | CC | 725 (98.4) | 503 (98.4) | 1.0 | 0.4-2.4 | 1.0 |
| | | CT | 12 (1.6) | 8 (1.6) | 1.0 | 0.4-2.6 | 1.0 |

CI – confidence interval; OR – odds ratio; p – p-value

variants of *RNASEL* were seen in similar frequency in cases and controls. We saw a trend towards a higher frequency of the P275A variant in unselected cases than in controls (10 vs. 7.2%; OR=1.4, p=0.1). A truncating *MSR1* R293X mutation was present in 1.6% of controls, and 1.6% of unselected cases.

## 1.2. Germline 657del5 mutation in the *NBS1* gene and susceptibility to prostate cancer

(based on: Cybulski C, Górski B, Debniak T, Gliniewicz B, Mierzejewski M, Masojć B, Jakubowska A, Matyjasik J, Złowocka E, Sikorski A, Narod SA, Lubiński J. NBS1 is a prostate cancer susceptibility gene. Cancer Res 2004; 64: 1215-1219)

Individuals with inherited recessive clinical syndromes, such as Nijmegen breakage syndrome (NBS), which is characterized by spontaneous chromosomal instability, immunodeficiency and a predisposition to cancer, carry a mutation in one of the genes in the DNA damage signalling pathway [20]. The product of the *NBS1* gene is responsible for DNA damage repair [21].

A 5-bp deletion in exon 6 of *NBS1* (657del5) is present in the majority of NBS patients from Eastern Europe [22]. This variant is present in approximately 0.6% of individuals (heterozygous carriers) from the general population in Poland [23, 24]. It has been suggested that heterozygous carriers of the founder mutation of NBS (657del5 allele) might be at increased risk of cancer [25, 26], but prostate cancer specifically has not been studied to date.

### Materials and methods

The case group consisted of 340 men diagnosed with prostate cancer at the University Hospital in Szczecin. Family histories of cancer were obtained from each subject. Thirty-five patients had one or more first- or second-degree relatives with prostate cancer (familial cases). We also included a second set of 21 familial cases of prostate cancer from men who were referred for evaluation at the Hereditary Cancer Centre by family doctors or urologists because of familial aggregation of prostate cancers. There were 1500 unaffected control subjects. One thousand control subjects were selected at random from the computerized patient lists of three family practices in Szczecin. A second control group comprised 500 newborns from Szczecin for whom a sample of umbilical cord blood was obtained.

Allele-specific PCR was used to detect the *NBS1* founder mutation in DNA isolated from peripheral blood leukocytes of cases and controls. A separate DNA sample was sequenced to confirm the presence of the *NBS1* mutation.

For the loss of heterozygosity (LOH) studies, for each of the nine prostate cancers in men with an *NBS1* mutation, a single non-carrier control tumour was selected. The control subject was born within 2 years of the patient and had a tumour of the same Gleason score as the matched patient. DNA was obtained from eight of the nine paraffin-embedded, microdissected tumours from *NBS1* mutation carriers and from all of the nine non-carrier control subjects. For the LOH analyses, two primer pairs were used, corresponding to the polymorphic microsatellite markers D8S88 and D8S1811. PCR was performed using fluorescent primers. PCR products were separated in an ABI PRISM 377 DNA Sequencer (Applied Biosystems). Data collection and analysis were performed using ABI PRISM 377 Collection Software and GenScan Analysis Software Version 3.0 (Applied Biosystems). A signal reduction in one allele of at least 70% was taken as the threshold of recognition for LOH. The *NBS1* mutant allele is five nucleotides shorter than the wild-type *NBS1* allele. For the LOH analysis of mutation-positive cases, additional primers were designed specifically to amplify exon 6 of *NBS1*, which contains the deleted sequence. PCR conditions using this primer set were as for allele-specific PCR. This primer set generates two distinct fragments from constitutional DNA from men with an *NBS1* deletion.

### Results

The *NBS1* mutation was present in 9 of 340 unselected patients with prostate cancer (2.6%) compared with only 9 of 1500 (0.6%) control subjects from the general population (OR=4.5; p=0.002). The 657del5 germline mutation was present in 5 of the 56 (9%) familial cases (OR=16; p<0.0001). We investigated the segregation of the *NBS1* mutant allele with prostate cancer in four families. We were able to establish the mutation status in two affected males from each family; in each family, the *NBS1* mutation was present in both affected members (Figure 1).

To analyze whether the wild-type allele of *NBS1* is lost in prostate cancer, we performed LOH analysis of microdissected prostate tumours from eight patients who carried the *NBS1* mutation and from nine patients who were found not to carry the *NBS1* mutation. The wild-type *NBS1* allele was lost in seven of eight prostate tumours from carriers of the 657del5 allele, but loss of heterozygosity was seen in only one of nine tumours from noncarriers (p=0.003).

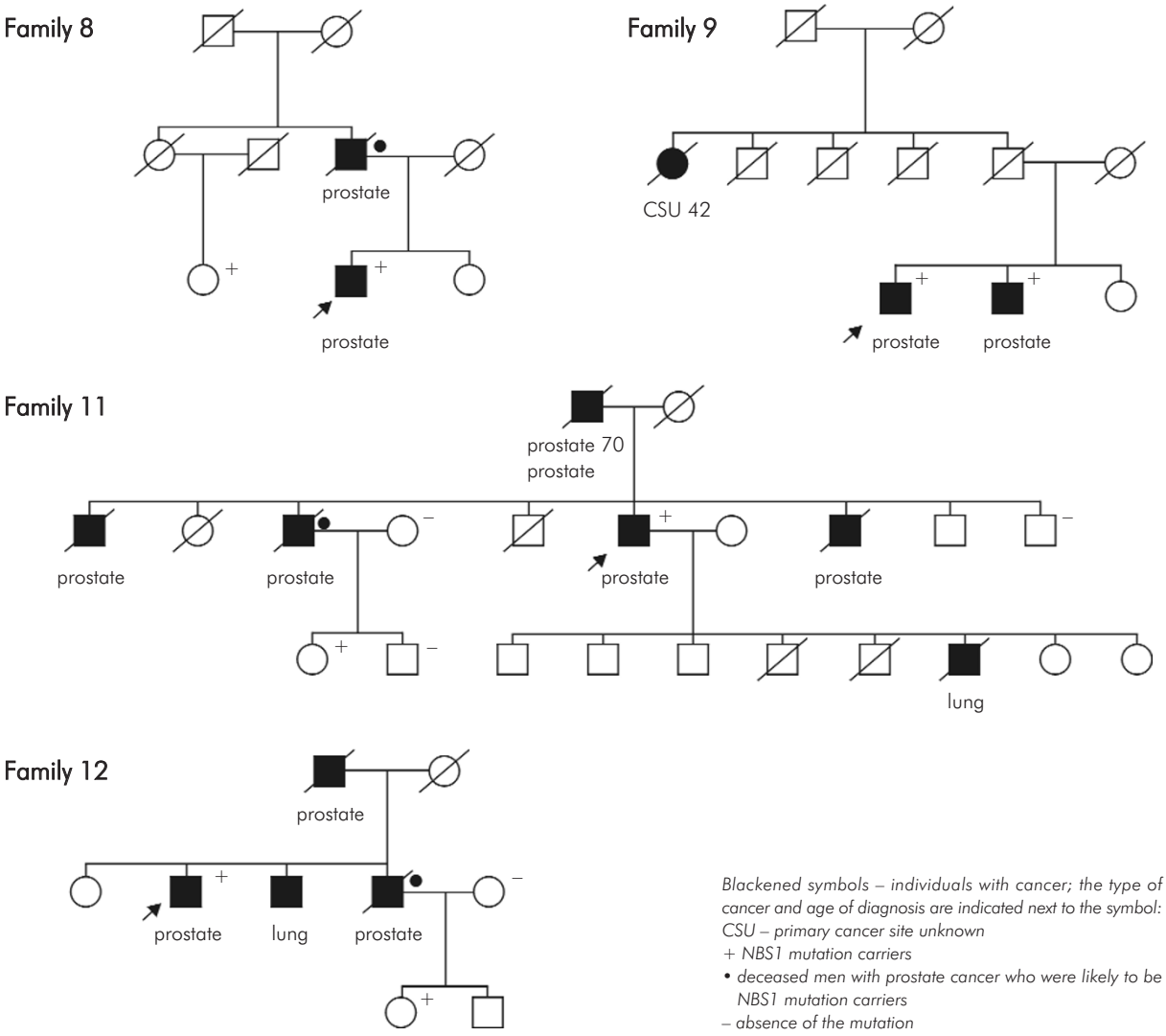



**Fig. 1.** Pedigrees of *NBS1* mutation positive cases with familial prostate cancer

## 1.3. Germline mutations in the *BRCA1* gene and susceptibility to prostate cancer

(on based: Cybulski C, et al. BRCA1 mutations and prostate cancer in Poland. Eur J Cancer Prev 2007 – in press)

*BRCA1* mutations confer high risk of breast and ovarian cancer [27]. Several studies suggested an increased risk of prostate cancer in Ashkenazi Jewish men with a *BRCA1* mutation (185delAG or 5382insC) [28-30]. Other studies, in non-Jewish populations, have found little or no evidence of an increased risk for prostate cancer in *BRCA1* carriers [31-34]. In Poland, there are three common founder alleles in *BRCA1* (C61G, 4153delA and 5382insC), which, in total, account for 90% of all *BRCA1* mutations [27]. Here we investigated if Polish men who carry one of these three alleles in *BRCA1* are at an increased risk of prostate cancer.

### Materials and methods

#### *Patients*

The case group consisted of 1793 unselected prostate cancer cases, collected in 13 centres situated throughout Poland (Szczecin, Bialystok, Olsztyn, Opole, Koszalin, Gdansk, Lublin, Lodz, Warszawa, Wroclaw,

**Table 2.** Comparison of the frequency of *BRCA1* mutations in 1793 patients with prostate cancer and 4570 controls

| Mutation | Number of carriers (frequency) | | OR | 95% CI | p |
|---|---|---|---|---|---|
| | cases (n=1793) No. (%) | controls (n=4570) No. (%) | | | |
| *BRCA1* | 8 (0.45) | 22 (0.48) | 0.9 | 0.4-2.1 | 1.0 |
| C61G | 3 (0.17) | 3 (0.07) | 2.6 | 0.5-12.7 | 0.5 |
| 4153delA | 4 (0.22) | 2 (0.04) | 5.1 | 0.9-27.9 | 0.1 |
| 5382insC | 1 (0.06) | 17 (0.37) | 0.15 | 0.02-1.1 | 0.06 |
| C61G or 4153delA* | 7 (0.39) | 5 (0.11) | 3.6 | 1.1-11.3 | 0.045 |

CI – confidence interval; OR – odds ratio; p – p-value
*when 5382insC is excluded, as unlikely pathogenic for prostate cancer in the Polish population

Poznan, Rzeszow, Sucha Beskidzka). Study subjects were unselected for age or family history. Family histories were obtained from each participant. Two hundred and twenty-nine patients had one or more first- or second-degree relative with prostate cancer (familial cases).

The control group consisted of a mix of 2000 newborn children from 10 hospitals throughout Poland (Szczecin, Bialystok, Gorzow, Katowice, Wroclaw, Poznan, Opole, Lodz and Rzeszow), 1570 adults selected at random from the patient lists of three family doctors practicing in the Szczecin region and 1000 individuals from Szczecin who submitted blood for paternity testing. In total there were 4570 population controls.

### *Methods*

The 4153delA and 5382insC mutations were detected using a multiplex-specific polymerase chain reaction (PCR) assay. The third mutation (C61G) generates a novel restriction enzyme site in exon 5. This mutation is detected after digesting amplified DNA with AvaII. All mutations were confirmed by sequencing.

### Results

A *BRCA1* mutation was seen in eight of 1793 (0.45%) cases and in 22 of 4570 (0.48%) controls (OR=0.9; p=1.0) – Table 2. 5382insC is the most frequent mutation of the three Polish founder mutations. The 5382insC mutation was detected only in 0.06% of cases, compared with 0.37% of controls (OR=0.15; p=0.06). In contrast, 4153delA was more common in cases than in controls (0.22 vs. 0.04%; OR=5.1; p=0.1). The C61G mutation was also more frequent in cases than in controls (0.17 vs. 0.07%; OR=2.6; p=0.5). A statistical test of homogeneity of the OR rejected the null hypothesis that the ORs associated with the three mutations were similar (p=0.008).

A *BRCA1* mutation was found in three of 229 (1.3%) familial prostate cancer cases, compared with five of 4570 controls (OR=12; 95% confidence interval (CI) 2.9–51; p=0.0004). The 4153delA mutation was present in one familial case (OR=10.0; p=0.3) and C61G was responsible for two other prostate cancer families (OR=13.4; p=0.008). The family with the 4153delA mutation contained two men with prostate cancer and the families with the C61G mutation contained four and five men with prostate cancer. The C61G segregated with prostate cancer in the two families (Figure 2).

## 2. Germline mutations in the *CHEK2* gene and their association with predisposition to prostate cancer and tumours of other sites of origin

### 2.1. Identification of point mutations in the *CHEK2* gene in the Polish population

(based on: Cybulski C, Huzarski T, Górski B, Masojć B, Mierzejewski M, Debniak T, Gliniewicz B, Matyjasik J, Złowocka E, Kurzawski G, Sikorski A, Posmyk M, Szwiec M, Czajka R, Narod SA, Lubiński J. A novel founder *CHEK2* mutation is associated with increased prostate cancer risk. Cancer Res 2004; 64: 2677-2679)

Germline mutations in the *CHEK2* gene have been described in several populations. For example, in the United States, 18 different *CHEK2* mutations were found [35]. Two founder variants in the *CHEK2* gene (1100delC and I157T) are present in Finland [36]. In the Ashkenazi Jewish population, a single S428F mutation was detected [37]. In order to identify *CHEK2* variants present in the

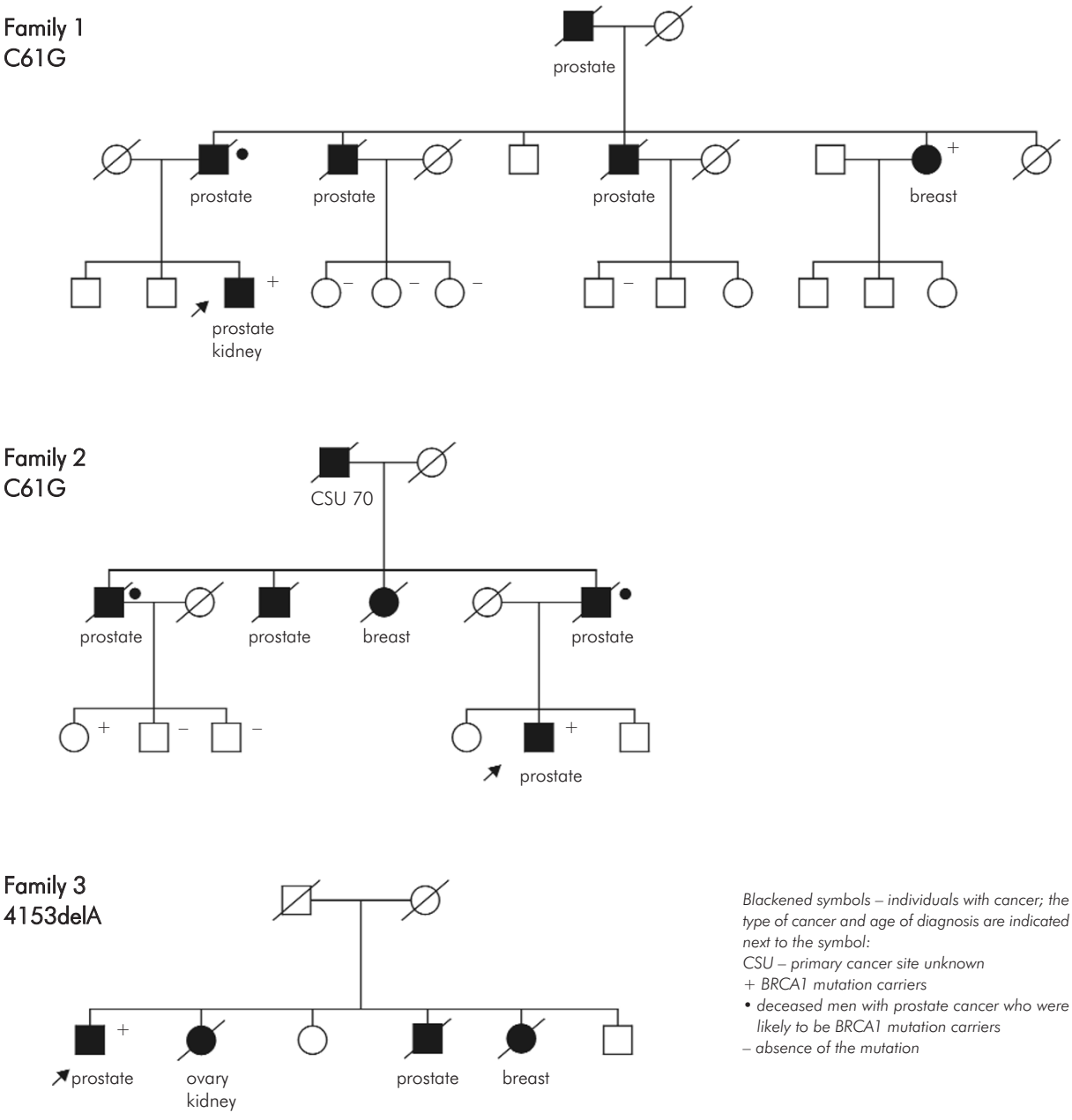



**Fig. 2.** Pedigrees of *BRCA1* mutation positive cases with familial prostate cancer

Polish population, we screened the entire coding *CHEK2* sequence in 140 men with prostate cancer.

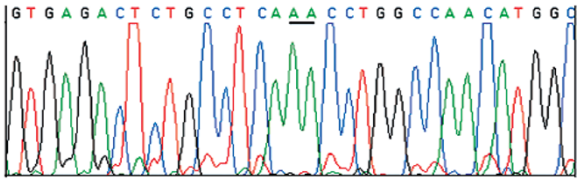
## Materials and methods

The case group consisted of 140 prostate cancer patients (including 44 familial cases). The entire coding region of the *CHEK2* gene was sequenced using primers and conditions described previously [35].

## Results

Three mutations were detected, the I157T missense variant and two truncating mutations IVS2+1G>A and 1100delC.

A.




B.

Telomeric breakpoint TGA GAC TCT GCC TCA **AA** AAA AAA AAA AAT A

Deletion sequence TGA GAC TCT GCC TCA **AA** CCT GGC CAA CAT G

Centromeric breakpoint CAG GAG TTT GAG ACC **AA** CCT GGC CAA CAT G

**Fig. 3.** A 5395 bp deletion of exons 9 and 10 of *CHEK2* detected in the Polish population: **A** – sequencing chromatogram of PCR product containing the deletion; **B** – location of deletion breakpoints on chromosome 22 in *Alu*-repeats (shown in bold)

## 2.2. Identification of large deletion of exons 9 and 10 of the *CHEK2* gene in the Polish population

(based on: Cybulski C, Wokołorczyk D, Huzarski T, Byrski T, Gronwald J, Górski B, Debniak T, Masojć B, Jakubowska A, Gliniewicz B, Sikorski A, Stawicka M, Godlewski D, Kwias Z, Antczak A, Krajka K, Lauer W, Sosnowski M, Sikorska-Radek P, Bar K, Klijer R, Zdrojowy R, Małkiewicz B, Borkowski A, Borkowski T, Szwiec M, Narod SA, Lubiński J. A large germline deletion in the *CHEK2* kinase gene is associated with an increased risk of prostate cancer. J Med Genet 2006; 43: 863-866)

Recently, a large deletion in *CHEK2* was identified in several unrelated patients with breast cancer of Czech or Slovak origin. Haplotype analysis confirmed that the mutation had a single source. The geographical and ethnic extent of this founder allele has not yet been determined [38]. We sought to establish if this deletion is present in the Polish population.

### Materials and methods

Three samples of pooled DNA, each including pooled DNA from about 500 people from Poland, were amplified with primers described previously for the detection of the deletion of exons 9 and 10 [38]. Ninety unpooled DNA samples representing individual controls were also included.

Short extension times (2 min) were applied during polymerase chain reaction (PCR) to amplify only a short allele containing the large deletion. A single PCR product of about 1.3 kb was amplified from all samples with the pooled DNA. The short product was seen in 1 of 90 DNA samples from single patients. The PCR products from all positive cases were sequenced.

### Results

A large deletion of exons 9 and 10 of the *CHEK2* gene (del5395) is also a founder mutation in Poland. A single PCR product of about 1.3 kb was amplified from all samples with the pooled DNA. The short product was seen in 1 of 90 DNA samples from single patients. The PCR products from all positive cases were sequenced. The deletion breakpoints were characterized at a nucleotide level (Figure 3). By our estimate, the length of this deletion is 5395 bp, and not 5567 bp as described in the original report [38].

## 2.3. *CHEK2* mutations and susceptibility to prostate cancer

(based on: Cybulski C, Wokołorczyk D, Huzarski T, Byrski T, Gronwald J, Górski B, Debniak T, Masojć B, Jakubowska A, Gliniewicz B, Sikorski A, Stawicka M, Godlewski D, Kwias Z, Antczak A, Krajka K, Lauer W, Sosnowski M, Sikorska-Radek P, Bar K, Klijer R, Zdrojowy R, Małkiewicz B, Borkowski A, Borkowski T, Szwiec M, Narod SA, Lubiński J. A large germline deletion in the *CHEK2* kinase gene is associated with an increased risk of prostate cancer. J Med Genet 2006; 43: 863-866)

*CHEK2* gene mutations have been associated with an increased prostate cancer risk in men from the United States [35] and Finland [39], but the Polish population has not been studied. In this study, we investigated whether *CHEK2* plays an important role in the development of prostate cancer in Poland.

### Materials and methods

We studied 1864 prostate cancer cases diagnosed between 1999 and 2005 in 13 centres situated throughout Poland. All the cases were unselected by age and family history. To estimate the frequency of the Polish founder mutations in the general population, three control groups were combined. The first group consisted of 2183 newborn children from 10 cities in Poland (Szczecin, Bialystok, Gorzow, Katowice, Wroclaw, Poznan, Opole, Lodz and Rzeszow) between 2003 and 2006. Samples of cord blood from unselected infants were forwarded to the study centre in Szczecin. The second control group included healthy adult patients (1079 women and 817 men) of three family doctors practicing in the Szczecin region. The third control group consisted of 1417 young adults (705 women and 712 men) from Szczecin who submitted blood for paternity testing.

Large deletion of exons 9 and 10 was genotyped in multiplex PCR reaction. The I157T and IVS2+1G>A

**Table 3.** Comparison of the frequency of *CHEK2* mutations in prostate cancer patients and in the control group

| Mutation | Group | Number of carriers/number of tested (frequency, %) | OR | 95% CI | p |
|---|---|---|---|---|---|
| del5395 | controls | 24/5496 (0.4) | 1.0 | | |
| | unselected cases | 15/1864 (0.8) | 1.9 | 0.97-3.5 | 0.009 |
| | familial cases | 4/249 (1.6) | 3.7 | 1.3-10.8 | 0.03 |
| 1100delC | controls | 12/5496 (0.2) | 1.0 | | |
| | unselected cases | 14/1864 (0.8) | 3.5 | 1.6-7.5 | 0.002 |
| | familial cases | 3/249 (1.2) | 5.6 | 1.6-19.9 | 0.02 |
| IVS2+1G>A | controls | 22/5496 (0.4) | 1.0 | | |
| | unselected cases | 15/1864 (0.8) | 2.0 | 1.05-3.9 | 0.052 |
| | familial cases | 5/249 (2.0) | 5.1 | 1.9-13.6 | 0.002 |
| Protein truncating mutation* | controls | 58/5496 (1.1) | 1.0 | | |
| | unselected cases | 44/1864 (2.4) | 2.3 | 1.5-3.4 | <0.0001 |
| | familial cases | 12/249 (4.8) | 4.7 | 2.5-9.0 | <0.0001 |
| I157T | controls | 264/5496 (4.8) | 1.0 | | |
| | unselected cases | 142/1864 (7.6) | 1.6 | 1.3-2.0 | <0.0001 |
| | familial cases | 30/249 (12.0) | 2.7 | 1.8-4.1 | <0.0001 |
| *CHEK2*** | controls | 321/5496 (5.8) | 1.0 | | |
| | unselected cases | 184/1864 (9.9) | 1.8 | 1.5-2.1 | <0.0001 |
| | familial cases | 42/249 (16.9) | 3.3 | 2.3-4.6 | <0.0001 |

CI – confidence interval; OR – odds ratio; p – p-value
*one of the three truncating mutations (del5395, IVS2+1G>A, 1100delC)
**any *CHEK2* mutation (del5395, IVS2+1G>A, 1100delC, I157T)

variants were analyzed by restriction fragment length polymorphism PCR. 1100delC was analyzed using an allele-specific PCR assay. Positive results were confirmed by sequencing.

## Results

Protein truncating mutations in the *CHEK2* gene (del5395, 1100delC, IVS2+1G>A) were more frequent in 1864 men with prostate cancer than in 5496 control individuals (2.4 vs. 1.1%; OR=2.3; p<0.0001). The I157T missense mutation was also more common in unselected cases than in controls (7.6 vs. 4.8%; OR=1.6; p<0.0001). The frequency of *CHEK2* mutations was also found to be higher in a series of 249 familial prostate cancer cases than in controls, both for truncating variants (4.8 vs. 1.1%; OR=4,7; p<0.0001) and the I157T missense variant (12.0 vs. 4.8%; OR=2.7; p<0.0001) (Table 3).

## 2.4. *CHEK2* mutations and susceptibility to breast cancer

(based on: Cybulski C, Wokołorczyk D, Huzarski T, Byrski T, Gronwald J, Górski B, Debniak T, Masojć B, Jakubowska A, van de Wetering T, Narod SA, Lubiński J. A deletion in *CHEK2* of 5,395 bp predisposes to breast cancer in Poland. Breast Cancer Res Treat 2007; 102:119-122)

A founder allele in *CHEK2*, 1100delC, has been reported to be a low-penetrance breast cancer susceptibility allele in several studies, and in many ethnic groups [37, 40-45]. Other *CHEK2* variants (IVS2+1G>A, I157T, and S428F) have also been suggested to confer increased breast cancer risks [44, 45]. Recently, a large deletion of exons 9 and 10 of

**Table 4.** Comparison of *CHEK2* mutation frequency in women with breast cancer and in controls

| Mutation | Group | Number of carriers/number of tested (frequency, %) | OR | 95% CI | p |
|---|---|---|---|---|---|
| del5395 | controls | 24/5496 (0.4) | 1.0 | | |
| | unselected cases | 19/1978 (1.8) | 2.2 | 1.2-4.0 | 0.01 |
| | early onset cases | 28/3229 (0.9) | 2.0 | 1.2-3.4 | 0.02 |
| 1100delC | controls | 12/5496 (0.2) | 1.0 | | |
| | unselected cases | 10/1978 (0.6) | 2.3 | 1.0-5.4 | 0.08 |
| | early onset cases | 16/3228 (0.5) | 2.3 | 1.1-4.8 | 0.04 |
| IVS2+1G>A | controls | 22/5496 (0.4) | 1.0 | | |
| | unselected cases | 21/1978 (1.1) | 2.7 | 1.5-4.9 | 0.002 |
| | early onset cases | 31/3228 (1.0) | 2.4 | 1.4-4.2 | 0.002 |
| Protein truncating mutation* | controls | 58/5496 (1.1) | 1.0 | | |
| | unselected cases | 49/1978 (2.5) | 2.4 | 1.6-3.5 | <0.0001 |
| | early onset cases | 74/3228 (2.3) | 2.2 | 1.6-3.1 | <0.0001 |
| I157T | controls | 264/5496 (4.8) | 1.0 | | |
| | unselected cases | 134/1978 (6.8) | 1.4 | 1.2-1.8 | 0.001 |
| | early onset cases | 207/3228 (6.4) | 1.4 | 1.1-1.6 | 0.002 |
| *CHEK2*** | controls | 321/5496 (5.8) | 1.0 | | |
| | unselected cases | 180/1978 (9.1) | 1.6 | 1.3-2.0 | <0.0001 |
| | early onset cases | 279/3228 (8.6) | 1.5 | 1.3-1.8 | <0.0001 |

CI – confidence interval; OR – odds ratio; p – p-value
*one of the three truncating mutations (del5395, 1100delC, IVS2+1G>A)
**any *CHEK2* mutation (del5395, 1100delC, IVS2+1G>A, I157T)

*CHEK2* was identified in two USA families at high risk of breast cancer [38]. The aim of the study was to establish the relationship between *CHEK2* mutations and the risk of breast cancer Poland.

## Materials and methods

This study included prospectively ascertained cases of invasive breast cancer diagnosed throughout Poland from 1996 to 2003. The case group consisted of two groups of women with breast cancer. The first group had 3228 cases diagnosed at age 50 or below. The second group included a sample of 1978 patients, unselected for age. Of these, 752 were under the age of 51 and were also enrolled in the early-onset study described above. All cases were unselected for family history. The control group consisted of 5496 patients. The controls are described in detail in section 2.3.

Genotyping methods are described in detail in section 2.3.

## Results

Protein truncating mutations (del5395, 1100delC, IVS2+1G>A) were observed more frequently in 1978 unselected breast cancer cases compared to controls (2.3 vs. 1.1%; $p<0.0001$). The frequency of missense variant I157T ($p=0.0001$) was also greater among unselected breast cancer cases than in the control group (6.8 vs. 4.8%; $p=0.001$). The odds ratio for breast cancer associated with truncating mutations (OR=2.4) was greater than that associated with the missense variant I157T (OR=1.4) – Table 4.

Protein-truncating mutations (del5395, 1100delC, IVS2+1G>A) were detected in 2.2% of 3228 patients with breast cancer diagnosed before the age of 51.

**Table 5.** Comparison of *CHEK2* mutation frequency in patients with colorectal cancer and controls

| Mutation | Group | Number of carriers/number of tested (frequency, %) | OR | 95% CI | p |
|---|---|---|---|---|---|
| Protein truncating mutation* | controls | 58/5496 (1.1) | 1.0 | | |
| | unselected cases | 11/1058 (1.0) | 1.0 | 0.5-1.8 | 0.9 |
| | familial cases | 2/110 (1.8) | 1.7 | 0.4-7.2 | 0.44 |
| I157T | controls | 264/5496 (4.8) | 1.0 | | |
| | unselected cases | 77/1085 (6.6) | 1.5 | 1.2-2.0 | 0.002 |
| | familial cases | 11/110 (10) | 2.2 | 1.2-4.1 | 0.01 |

CI – confidence interval; OR – odds ratio; p – p-value
*one of the three truncating mutations (del5395, 1100delC, IVS2+1G>A)

This frequency was significantly higher ($p<0.0001$) than in the control group (1.1%). The missense variant I157T was also significantly more common ($p=0.002$) among patients with early onset breast cancer (6.4%) than in controls (4.8%). The odds ratio for early onset breast cancer associated with a truncating *CHEK2* mutation (OR=2.2) was greater than that associated with the I157T mutation (OR=1.4) – Table 4.

## 2.5. *CHEK2* mutations and susceptibility to colorectal cancer

(based on: Cybulski C, Wokołorczyk D, Kładny J, Kurzawski G, Suchy J, Grabowska E, Gronwald J, Huzarski T, Byrski T, Górski B, D Ecedil Bniak T, Narod SA, Lubiński J. Germline *CHEK2* mutations and colorectal cancer risk: different effects of a missense and truncating mutations? Eur J Hum Genet 2007; 15: 237-241)

Germline mutations in *CHEK2* have been associated with a range of cancer types, in particular of the breast and the prostate [35-47]. Protein-truncating mutations in *CHEK2* have been reported to confer higher risk of cancer of the breast and the prostate than the missense I157T variant. Recent studies from Finland suggest an increased risk of colon cancer among carriers of missense variant I157T, but not in carriers of *CHEK2* protein truncating mutations [36].

The aim of the study was to evaluate the association between specific *CHEK2* alleles and colon cancer.

### Materials and methods

We studied 1085 colorectal cancer cases diagnosed between 1998 and 2005 in three centres in North-Western Poland. Patients were unselected for age and family history. 964 colon cancer cases were diagnosed in Szczecin and 121 cases were diagnosed in Koszalin and Kolobrzeg. One hundred and ten cases had first-degree relatives diagnosed with colon cancer (familial cases). The control group consisted of 5496 patients. The controls are described in detail in section 2.3. Genotyping methods are described in detail in section 2.3.

### Results

The missense mutation I157T was overrepresented both in unselected cases (6.6%) and familial cases (10%) with colorectal cancer compared to controls (4.8%). The OR for unselected colorectal cancer cases with the missense mutation I157T was 1.5 ($p=0.002$) – Table 5. The OR for familial cases with the I157T variant was 2.2 ($p=0.01$). We saw no association between colorectal cancer risk and *CHEK2* truncating alleles.

## 2.6. *CHEK2* mutations and susceptibility to tumours of other sites of origin

(based on: Cybulski C, Górski B, Huzarski T, Masojć B, Mierzejewski M, Debniak T, Teodorczyk U, Byrski T, Gronwald J, Matyjasik J, Zlowocka E, Lenner M, Grabowska E, Nej K, Castaneda J, Medrek K, Szymańska A, Szymańska J, Kurzawski G, Suchy J, Oszurek O, Witek A, Narod SA, Lubiński J. *CHEK2* is a multiorgan cancer susceptibility gene. Am J Hum Genet 2004; 75: 1131-1135)

*CHEK2* mutations originally were found in families with Li-Fraumeni syndrome [46, 47]. The *CHEK2* protein is expressed in a wide range of tissues and participates in the DNA damage response in many cell types; therefore *CHEK2* is a good candidate for a multi-site cancer susceptibility gene.

The aim of the study was to assess the range of cancers associated with inactivating mutations in the *CHEK2* gene.

### Materials and methods

To establish the range of cancer types associated with *CHEK2* mutations, we genotyped unselected cases of cancer with the most common types of cancer in Poland. In this part the results of 2001 cancer patients are described (excluding cases of the prostate, breast and colon cancers, which are described in detail in previous sections).

The control group consisted of: 2 000 newborn children from 10 hospitals throughout Poland (Szczecin, Bialystok, Gorzow, Katowice, Wroclaw, Poznan, Opole, Lodz and Rzeszow); 1000 adult patient lists of three family doctors practicing in the Szczecin region; 1000 adults unselected from family history from Szczecin who submitted blood for paternity testing.

Genotyping methods are described in detail in section 2.3. To confirm the chromosomal location of the observed *CHEK2* mutations, we analyzed the polymorphic marker *D22S275*, which maps to intron 4 of *CHEK2*. We genotyped: 36 patients with the I157T variant; 24 patients with 1100delC; 52 patients with IVS2+1G>A and 50 individuals from the general population.

### Results

The frequencies of the three *CHEK2* variants in cases and controls are presented in Table 6. Because of their different effects on protein synthesis, the two truncating mutations (IVS2+1G>A or 1100delC) were considered separately from the missense mutation (I157T).

*CHEK2* truncating alleles were associated with increased risk of breast and prostate cancer (described in the previous sections) and thyroid cancer (OR=4.9; p=0.0006). The missense variant I157T was associated with an increased risk of prostate, breast and colon cancer (described in the previous sections) and in addition with an increased risk of kidney cancer (OR=2.0; p=0.0006) and thyroid cancer (OR=1.9; p=0.04).

Although any individual finding might be due to chance, our study on the whole suggests that mutations in *CHEK2* increase the risk of cancer in many different organs. A total of 52 comparisons were made. Of these, 13 were significant at the p=0.05 level (2.6 expected by chance) and 5 were significant at the p=0.01 level (0.5 expected). Furthermore, in all three sites for which a significant association was seen with the truncating mutation, a significant association was also seen with the missense mutation. This would be unlikely to be the case if the observations were due to chance.

To confirm the chromosomal location of the observed *CHEK2* mutations we analyzed the polymorphic marker *D22S275*, which maps to intron 4 of *CHEK2*. All individuals with the 1100delC variant or with the I157T variant carried the 165 bp allele of D22S275, which we estimate has a frequency of 15% in the Polish population. All individuals with the IVS2+1G>A mutation carried the 171bp allele of the D22S275 marker, which we estimate has a frequency of 8% in the general population. These observations support the chromosome 22 assignment for the three variant alleles.

## 2.7. Clinical characteristics of *CHEK2*-positive breast cancers in young women from Poland

(based on: Cybulski C, Górski B, Huzarski T, Byrski T, Gronwald J, Debniak T, Wokolorczyk D, Jakubowska A, Kowalska E, Oszurek O, Narod SA, Lubinski J. *CHEK2*-positive breast cancers in young Polish women. Clin Cancer Res 2006; 12: 4832-4835)

A founder allele in *CHEK2*, 1100delC, has been reported to be a low-penetrance breast cancer susceptibility allele in several studies, and in many ethnic groups. Recent studies reported that patients with the *CHEK2* 1100delC variant had a 6-times higher risk of contralateral breast cancer and 3-times higher risk of distant metastasis [42, 48, 49] and more frequently developed ER-positive tumours than non-carriers (91 vs. 69%; p=0.03) [48]. Although the relationship between *CHEK2* mutations and breast cancer is well documented, little is known about clinical characteristic of cancers in carriers of *CHEK2* mutations.

To investigate the contribution of *CHEK2* mutations to early-onset breast cancer in Poland, and to establish the characteristic features of these cancers, we compared clinical and pathological features of *CHEK2*-positive and *CHEK2*-negative breast cancers.

### Materials and methods

The case group consisted of 3228 patients with breast cancer diagnosed before age 50, unselected from family history, who tested negative for *BRCA1* mutation (4153delA, 5328insC, and C61G). The medical and pathology reports of the cases were reviewed locally by the physician associated with the study and relevant information was forwarded to the study centre in Szczecin. Information was recorded on age at diagnosis, stage, grade and lymph node status, oestrogen-receptor status, multicentricity and bilaterality. The data were collected from at least 70% of patients. Tumour blocks and/or paraffin-embedded slides were requested from the corresponding pathology centres. A central pathology review of was conducted in Szczecin by two pathologists associated with the study.

**Table 6.** Comparison of *CHEK2* mutation frequency in patients with selected tumours and in the control group

| Location or type of tumour | Number of tested | Number of mutation carriers (frequency), results of statistical analysis* | | | |
|---|---|---|---|---|---|
| | | IVS2+1G>A | 1100delC | 1100delC or IVS2+1G>A | I157T |
| Bladder | 172 | 1 (0.6%) OR=1.2 p=0.7 | 0 | 1 (0.6%) OR=0.8 p=0.8 | 12 (7.0%) OR=1.5 p=0.3 |
| Kidney | 264 | 0 | 2 (0.8%) OR=2.7 p=0.5 | 2 (0.8%) OR=1.0 p=0.8 | 26 (9.8%) OR=2.1 p=0.0006 |
| Larynx | 245 | 0 | 0 | 0 | 10 (4.1%) OR=0.8 p=0.7 |
| Lung | 272 | 0 | 0 | 0 | 7 (2.6%) OR=0.5 p=0.1 |
| Melanoma | 129 | 2 (1.5%) OR=3.3 p=0.3 | 1 (0.8%) OR=3.1 p=0.8 | 3 (2.3%) OR=3.2 p=0.1 | 6 (4.6%) OR=1.0 p=0.9 |
| Ovary | 292 | 0 | 0 | 0 | 14 (4.8%) OR=1.0 p=0.9 |
| Stomach | 241 | 4 (1.7%) OR=3.5 p=0.05 | 0 | 4 (2.1%) OR=2.3 p=0.2 | 13 (5.4%) OR=1.1 p=0.8 |
| NHL | 120 | 1 (0.8%) OR=1.8 p=0.9 | 0 | 1 (0.8%) OR=1.1 p=0.7 | 11 (9.2%) OR=2.0 p=0.05 |
| Pancreas | 93 | 0 | 0 | 0 | 6 (6.4%) OR=1.4 p=0.6 |
| Thyroid | 173 | 5 (2.9%) OR=6.2 p=0.0003 | 1 (0.6%) OR=2.3 p=0.9 | 6 (3.5%) OR=4.9 p=0.0006 | 15 (8.7%) OR=1.9 p=0.04 |
| Controls | 4000 | 19 (0.475%) | 10 (0.25%) | 29 (0.725%) | 193 (4.825%) |

*comparison of *CHEK2* mutation frequency in patients with specific tumour type to that of control group
NHL – non-Hodgkin lymphoma; OR – odds ratio; p – p-value

## Results

A *CHEK2* mutation was identified in 252 of 3,228 women with breast cancer (7.8%), including I157T (207 times), IVS2+1G>A (31 times), and 1100delC (16 times). The mean age of diagnosis in women with a *CHEK2* mutation was similar to that of the non-carrier cases. However, the mean age of diagnosis of women with a truncating mutation was 1.8 years lower than women without a *CHEK2* mutation (42.5 vs. 44.3 years; p=0.01).

The characteristics of the breast cancer cases in the 252 women with a *CHEK2* mutation are presented in Table 7 and compared with non-carriers. Breast cancers in women with a *CHEK2* mutation were more commonly of lobular histology (21.5 vs. 15.8%; p=0.05), of size greater than 2 cm (54.8 vs. 43.5%; p=0.01) or of multi-centric origin (28.7 vs. 19.5%; p=0.01) than were cancers from women without a *CHEK2* mutation. Intraductal cancers (DCIS) with micro-invasion were also more common in women with a *CHEK2* mutation than in non-carriers (11.3 vs.

**Table 7.** Comparison of breast cancers in patients with *CHEK2* mutations to cancers in patients without *CHEK2* mutations

| Feature | | *CHEK2*-positive cases (n=252) | *CHEK2*-negative cases (n=2976) | p |
|---|---|---|---|---|
| Age in years (mean) | | 44.2 | 44.3 | 0.7 |
| Age group | 20-30 | 3.6% (9/252) | 1.7% (52/2976) | 0.07 |
| | 31-40 | 15.9% (40/252) | 17.2% (512/2976) | 0.7 |
| | 41-50 | 80.5% (203/252) | 81% (2412/2976) | 0.9 |
| Histology | ductal G1–2 grade | 29.3% (54/186) | 26.9% (622/2315) | 0.6 |
| | ductal G3 grade | 10.7% (20/186) | 12.7% (294/2315) | 0.5 |
| | medullary | 2.1% (4/186) | 4.9% (113/2315) | 0.1 |
| | lobular | 21.5% (40/186) | 15.8% (366/2315) | 0.05 |
| | tubulo-lobular | 4.3% (8/186) | 3.6% (83/2315) | 0.8 |
| | DCIS | 11.3% (21/186) | 7.2% (168/2315) | 0.06 |
| | other | 4.8% (9/186) | 5.1% (118/2315) | 1.0 |
| | missing or unknown | 16.7% (31/186) | 23.8% (551/2315) | 0.03 |
| Pre-operative chemotherapy | | 27.7% (66/238) | 24.5% (661/2693) | 0.3 |
| Oestrogen receptor | positive | 65.1% (97/149) | 63.7% (1048/1646) | 0.8 |
| Tumour size (cm) | <1 cm | 5.9% (9/152) | 11.2% (193/1728) | 0.05 |
| | 1-2 cm | 40.1% (61/152) | 45.3% (783/1728) | 0.2 |
| | >2 cm | 53.9% (82/152) | 43.5% (752/1728) | 0.01 |
| Lymph nodes | positive | 45.0% (68/151) | 40.1% (722/1777) | 0.3 |
| Multicentric | | 28.7% (41/143) | 19.5% (316/1619) | 0.01 |
| Bilateral | | 2.3% (5/215) | 3.3% (84/2531) | 0.6 |
| Family history positive* | | 13.8% (31/224) | 8.9% (237/2652) | 0.02 |

*family history refers to a first-degree relative of a proband affected with breast cancer
DCIS – intraductal cancer (ductal carcinoma in situ) with microinvasion; p – p-value

7.2%, p=0.06), but this difference was not significant. Carriers and non-carriers were similar with respect to oestrogen receptor status (65.1 vs. 63.7%; p=0.8) and lymph node status (45 positive vs. 40.1%; p=0.3). Bilateral tumours were equally common in both subgroups (2.3 vs. 3.3%; p=0.6).

The great majority of women with a *CHEK2* mutation did not have a strong family of cancer – 13.8% of the women with breast cancer and a *CHEK2* mutation were from a family with two or more first-degree relatives with breast cancer. However, this was more frequent than reported by the non-carriers (8.9%) and the difference was statistically significant (OR=1.6; p=0.02).

## 3. Summary of the results

1. The entire coding region of the *RNASEL* gene was sequenced in 94 men with familial prostate cancer and the coding region of the *MSR1* gene was screened in 52 men with familial prostate cancer. Four DNA variants were detected including R462Q and D541E in the *RNASEL* gene, and P275A and R293X in the *MSR1* gene. These variants were genotyped in a series of 737 unselected prostate cancer cases and 511 controls, but no significant differences in the allele frequencies were observed.
2. The *NBS1* 657del5 mutation was present in 2.6% of 340 unselected patients with prostate cancer

compared with only 0.6% of 1500 control subjects from the general population (OR=4.5; p=0.002). The 657del5 germline mutation was present in 9% of 56 men with familial prostate cancer (OR=16; p<0.0001). The 657del5 mutation segregated with disease in four families with familial prostate caner. Loss of the wild type *NBS1* allele was observed in seven of eight tumours from men who carried a germline *NBS1* deletion compared with only one of nine *NBS1* mutation-negative prostate tumours (p=0.003).

3. The 5382insC mutation in the *BRCA1* gene was detected only in one of 1793 prostate cancer cases (0.06%), whereas it was seen in 0.37% of 4570 controls (p=0.06). In contrast, the 4153delA and C61G mutations were found in excess in cases (0.39%) compared to controls (0.11%). The presence of either of these alleles (C61G or 4153delA) was associated with an increased risk for prostate cancer (OR=3.6; p=0.045), in particular for familial prostate cancer (OR=12; p=0.0004). Segregation analysis suggested that the C61G mutation segregated with disease in two families with familial prostate cancer. A statistical test of homogeneity of the odds ratio revealed that the risks associated with the three *BRCA1* mutations were different (p=0.008).
4. Three point mutations in the *CHEK2* gene (1100delC, IVS2+1G>A, I157T) were detected by sequencing of DNA isolated from peripheral blood of 140 men with prostate cancer. Large germline deletion of 5395 base pairs in length removing exons 9 and 10 of the *CHEK2* gene (del5395) was detected by analysis of samples with pooled DNA, each one including DNAs from approximately 500 individuals from Poland.
5. Protein truncating mutations in the *CHEK2* gene (del5395, 1100delC, IVS2+1G>A) were more frequent in 1864 men with prostate cancer than in 5496 control individuals (2.4 vs. 1.1%; OR=2.3; p<0.0001). The I157T missense mutation was also more common in unselected cases than in controls (7.6 vs. 4.8%; OR=1.6; p<0.0001). The frequency of *CHEK2* mutations was also found to be higher in a series of 249 familial prostate cancer cases than in controls, both for truncating variants (4.8 vs. 1.1%; OR=4.7; p<0.0001) and the I157T missense variant (12.0 vs. 4.8%; OR=2.7; p<0.0001).
6. Comparison of the frequency of *CHEK2* variants in 7540 patients (4454 with breast cancer, 1085 with colorectal cancer, 2001 with other tumours excluding prostate cancer) to that in controls from the general population revealed a positive association of *CHEK2* mutations with malignancies of different site of origin. *CHEK2* truncating alleles were associated with an increased risk of cancer development in the thyroid (OR=4.9; p=0.0006) and the breast (OR=2.4; p=0.0001). The missense variant I157T was associated with an increased risk of breast cancer (OR=1.4; p=0.001), colon cancer (OR=1.5; p=0.002), kidney cancer (OR=2.1; p=0.0006) and thyroid cancer (OR=1.9; p=0.04). The range of cancers associated with I157T missense variant of the *CHEK2* gene was greater than that associated with *CHEK2* truncating alleles.
7. Evaluation of 3228 unselected breast cancers diagnosed under the age of 51 years revealed that breast cancers in women with a *CHEK2* mutation were more commonly of lobular histology (21.5 vs. 15.8%; p=0.05), of size greater than 2 cm (54.8 vs. 43.5%; p=0.01) or of multi-centric origin (28.7 vs. 19.5%; p=0.01) than were cancers from women without a *CHEK2* mutation. In addition, mutation carriers more frequently had a positive family history of breast cancer than non-carriers (13.8 vs. 8.9%; p=0.02). Carriers and non-carriers were similar with respect to bilaterality, nodal status and oestrogen receptor status.

## 4. General conclusions

Groups of individuals with an increased risk of prostate cancer in the Polish population can be identified by testing of specific variants in the *NBS1*, *BRCA1* and *CHEK2* genes. It seems that analysis of the *RNASEL* and *MSR1* genes is not justified for this purpose. The list of known genetic markers of high risk of prostate cancer (in addition to strong family history of prostate cancer and germline mutations in the *BRCA2* gene) may be extended by specific mutations in the *NBS1*, *BRCA1* and *CHEK2* genes in men with a positive family history of prostate cancer in at least one 1$^{st}$ or 2$^{nd}$ degree relative (the risk increased about 5-15 fold).

*CHEK2* is multi-organ cancer susceptibility gene. It seems justified to consider surveillance of the prostate, breast, thyroid, kidney and colon as an option for carriers of *CHEK2* mutations. It appears reasonable to consider magnetic resonance imaging of the breast beginning from the age of ~35 as an option for mutation carriers, as *CHEK2* mutations may predispose to lobular and multifocal cancers in young women. However, the establishment of surveillance protocols for carriers of a *CHEK2* mutation requires further studies.

Most carriers of *NBS1* and *CHEK2* mutations report negative family history of prostate, breast and colorectal cancers. The use of DNA tests is the only

way to find carriers of these changes conferring increased risk of tumour development. Identification of low penetrance DNA variants may be very important if the simultaneous presence of such alternations and/or combinations with external risk factors in a carrier would add up to a clinically significant high risk of tumour development.

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