Advances in Brief

A Novel Founder CHEK2 Mutation is Associated with Increased Prostate Cancer Risk

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Abstract

Variants in the *CHEK2* have been found to be associated with prostate cancer risk in the United States and Finland. We sequenced *CHEK2* gene in 140 Polish patients with prostate cancer and then genotyped the three detected variants in a larger series of prostate cancer cases and controls. *CHEK2* truncating mutations (IVS2 + 1G>A or 1100delC) were identified in 9 of 1921 controls (0.5%) and in 11 of 690 (1.6%) unselected patients with prostate cancer [odds ratio (OR) = 3.4; $P = 0.004$]. These mutations were found in 4 of 98 familial prostate cases (OR = 9.0; $P = 0.0002$). The missense variant I157T was also more frequent in men with prostate cancer (7.8%) than in controls (4.8%), but the relative risk was more modest (OR = 1.7; $P = 0.03$). I157T was identified in 16% of men with familial prostate cancer (OR = 3.8; $P = 0.00002$). Loss of the wild-type *CHEK2* allele was not observed in any of prostate cancers from five men who carried *CHEK2*-truncating mutations. Our results provide evidence that the two truncating mutations of *CHEK2* confer a moderate risk of prostate cancer in Polish men and that the missense change appears to confer a modest risk.

Introduction

The genetic basis of prostate cancer is complex and appears to involve multiple genes. Three candidate susceptibility genes have been identified through linkage analysis (*HPC1*, *HPC2/ELAC2*, and *MSR1*), but a clear role for any of these genes in hereditary prostate cancer has not been established (1–6). Additionally, mutations in *BRCA2* and *NBS1* also predispose to prostate cancer (7–10), but the contribution of these genes to prostate cancer is relatively small.

CHEK2 [CHEK2, also known as "CHK2" (MIM 604373)] is located on chromosome 22q and encodes the human analog of yeast Cds1 and Rad53, which are checkpoint kinases (11, 12). Activation of these proteins in response to DNA damage prevents cellular entry into mitosis. *CHEK2* is activated through phosphorylation by ataxia telangiectasia mutated (ATM) protein in response to DNA damage induced by ionizing radiation (12–14). Activated *CHEK2* phosphorylates BRCA1 and TP53 proteins, regulating tumor suppressor function of these proteins (15–17). A founder variant in *CHEK2*, 1100delC was originally described in Li-Fraumeni families (18, 19) and as a low penetrance breast cancer susceptibility allele (20–22). Recently, *CHEK2* mutations have also been identified in patients with prostate cancer (23, 24). In this study, we investigated whether *CHEK2* plays an important role in the development of prostate cancer in the Polish population.

Materials and Methods

The case group consisted of 690 prostate cancer patients (mean age at onset, 67.6), including 377 consecutive patients with prostate cancer diagnosed in northwestern Poland between 1999 and 2003 (Szczecin), 248 unselected men with prostate cancer diagnosed between June 2002 to October 2003 in northeastern Poland (Olsztyn and Białystok), and 65 men with prostate cancer diagnosed in 2003 in southwest Poland (Opole). Ninety-eight patients (14.2%) had a first- or second-degree relative affected with prostate cancer (familial prostate cancer). They contained, on average, 2.2 cases of prostate cancer per pedigree (mean age of onset 67.1 years). The control population consisted of 500 consecutive newborns from the clinical hospitals of Szczecin, and 1421 controls selected at random from the computerized patient lists of five family practices in Szczecin, Białystok, and Olsztyn (725 females and 696 males).

DNA samples were obtained from peripheral blood of individuals or from umbilical cord blood of newborns. The entire coding region of *CHEK2* gene was sequenced in 96 men with sporadic prostate cancer and in 44 additional familial prostate cancer cases, using primers and conditions described previously (23). Exon 10 was screened with primers used in allele-specific PCR for the 1100delC as described below. Samples were sequenced with BigDye Terminator Ready Reaction kit and analyzed in ABI PRISM 377 DNA sequencer (Applied Biosystems).

Three detected sequence variants were then analyzed in a larger series of cases and controls. The 430T>C variant (Ile157Thr) was analyzed by restriction fragment length polymorphism PCR, using Ch157F (5′- ACCCATG-TATCTAGGAGAGCTG) and Ch157R (5′-CCACTGTGATCTTCTATGTC-TGCA) primers. The reverse primer introduced artificial restriction site for *Pst*I enzyme. The PCR products were digested in mutation-positive cases. The IVS2 + 1G>A mutation was identified by RFLP-PCR using Hpy 188III (New England Biolabs) and primers Ch2/3f (5′-ATTTATGAGCAATTTTTA-AACG) and Ch2/3r (5′-TCCAGTAACCATAAGATAATAATATTAC). PCR product was digested in cases with mutation. The 1100delC was analyzed using an allele-specific PCR assay using primers Chk2ex10f (5′-TTAATTTA-AGCAAAATTAAATGTC), Chk2ex10r (5′-GGCATGGTGGTGTGCATC), and Chk2delC (5′-TGGAGTGCCCAAAATCATA). PCR products were separated in 2–3% agarose gels. In cases positive in RFLP-PCR and allele-specific oligonucleotide-PCR analyses, DNA sample was sequenced to confirm the presence of the mutation. Odds-ratios and confidence limits were generated using Mantel-Haenszel χ^2 test.

Loss of heterozygosity analysis was performed with primer pair corresponding to the *CHEK2* intragenic microsatellite marker D22S275 in microdissected tumors of five mutation-positive men (one carrier of the 1100delC and four carriers of the IVS2 + 1G>A mutation) using methodology described in detail previously (10).

Results and Discussion

The frequencies of the three detected variants are presented in Table 1. One of the two truncating mutations (IVS2 + 1G>A and 1100delC) was detected in 11 of 690 (1.6%) unselected patients

Table 1 *Association of the IVS2+1G>A, 1100delC, and 1157T variants of CHEK2 gene with prostate cancer risk*

Variant and case	No. of carriers/total (frequency)	OR[a]	95% CI	P
IVS2+1G>A				
Controls	5/1921 (0.3%)	1.0		
Unselected cases	8/690 (1.2%)	4.5	1.5–13.8	0.004
Familial cases	3/98 (3.1%)	12.1	2.8–51.4	0.0002
1100delC				
Controls	4/1921 (0.2%)	1.0		
Unselected cases	3/690 (0.4%)	2.1	0.5–9.4	0.32
Familial cases	1/98 (1.0%)	4.9	0.5–44.6	0.11
Any truncating mutation[b]				
Controls	9/1921 (0.5%)	1.0		
Unselected cases	11/690 (1.6%)	3.4	1.4–8.3	0.004
Familial cases	4/98 (4.1%)	9.0	2.7–29.9	0.00001
I157T				
Controls	29/600 (4.8%)	1.0		
Unselected cases	54/690 (7.8%)	1.7	1.05–2.7	0.03
Familial cases	16/98 (16%)	3.8	2.0–7.4	0.00002

[a] OR, odds ratio; CI, confidence interval
[b] The IVS2+1G>A or 1100delC

compared with 9 of 1921 (0.5%) controls (OR = 3.4; $P = 0.004$). A mutation was identified in 4 of 98 (4.1%) familial cases (OR = 9.0; $P = 0.0001$). The missense variant I157T was also more frequent in men with prostate cancer (7.8%) than in controls (4.8%; OR = 1.7; $P = 0.03$). It was identified in 16% of men with familial prostate cancer (OR = 3.8; $P = 0.00002$).

In contrast to the situation in North America and Western Europe, the IVS2 + 1G>A is the dominant *CHEK2*-truncating mutation in Poland occurring with frequency of 0.3% in the general population compared with 0.2% for the 1100delC (which is the common mutation in western Europe). Haplotype analysis confirmed that this is a founder mutation (data not shown). The IVS2 + 1G>A variant has been reported previously in a single family with prostate cancer in the United States. The mutation creates a 4-bp insertion attributable to abnormal splicing, creates premature protein termination codon in exon 3, and leads to the disruption of protein expression (24). However, it is not clear if the IVS2 + 1G>A is a founder mutation particular to the Polish population or if it has been missed by other investigators who might have used less sensitive screening techniques (primarily conformation-sensitive gel electrophoresis; Refs. 24–27). We have identified the IVS2 + 1G>A allele in single cancer patients from Latvia and Belarus (unpublished data), suggesting that this allele is present in other Slavic populations. The other two *CHEK2* variants (1100delC and I157T) reported in our population were also reported among individuals from other ethnic groups (20–35).

The IVS2 + 1G>A and 1100delC truncating mutations are less common in Poland (0.5% of the general population) than the I157T missense change (4.8%), but the relative risk for prostate cancer associated with either of the two truncating mutations was higher (3.4 *versus* 1.7). We estimate that these mutations account for about 1% of prostate cancer cases in Poland, compared with 3% for the I157T missense variant. Our results support the hypothesis that the I157T allele confers increased susceptibility to prostate cancer. Combining data from the three studies (this study and Refs. 23 and 24), the I157T change was seen in 6.2% of 1627 cases *versus* 4.0% of controls (OR = 1.6; $P = 0.005$). It appears therefore that this missense variant is pathogenic for prostate cancer but confers lower penetrance than the truncating mutation. This variant does not appear to increase the risk of breast cancer (26, 27).

In our study, each of four familial prostate cancer families with the truncating mutation included two men with prostate cancer. Recently, variants in *CHEK2* gene (1100delC and I157T) were shown to associate with familial prostate cancer in Finland and similarly, a few men with prostate cancer were observed in mutation-positive Finish famil-

ial prostate cancer families (24). It is interesting that Dong *et al.* (23) reported an association with *CHEK2* mutations (18 different mutations pooled together) and sporadic prostate cancer but not familial prostate cancer. The failure to detect a significant association between *CHEK2* variants and familial cases could be attributable to the fact that the families in the United States study included a relatively high number of affected men (at least three affected in at minimum two generations).

We identified one of three different *CHEK2* alterations in 20 of 98 (20.4%) patients with familial prostate cancer. Seppala *et al.* (24) found one of three variants (1100delC, I157T, D438Y) in 17 of 120 (14.2%) familial prostate cases from Finland. In a study of 400 men with sporadic cancer and 298 men with familial prostate cancer from the United States, 18 different *CHEK2* mutations were found, mostly in single patients (23). In our study, only three pathogenic *CHEK2* variants were detected in 140 men who were fully sequenced. This reduced level of genetic variation is likely the result of the relative ethnic homogeneity in Poland.

Acknowledgments

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