Epistatic Relationship between the Cancer Susceptibility Genes CHEK2 and p27

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Abstract

We studied the effects of p27 and CHEK2 variants on prostate and colon cancer risk in a case-control study. Modest effects on prostate cancer risk were observed for both CHEK2 missense and truncating variants. However, the excess cancer risk was restricted to the subgroup of men who were homozygous for the VV genotype in codon 109 of the p27 gene. Among men with the VV p27 genotype, the odds ratios associated with truncating and missense CHEK2 mutations were 3.1 (P < 0.0001) and 1.9 (P < 0.0001), respectively. Among men with other p27 genotypes (GG and VG), the odds ratios were 1.5 and 1.2 for truncating and missense CHEK2 mutations, respectively, and were not statistically significant. The interaction between CHEK2 and p27 was confirmed in a group of patients with colon cancer. Thus, it seems that the clinical expression of CHEK2 variant alleles on prostate and colon cancer risk may be restricted to individuals with a specific genotype (VV) of the p27 gene. Two-gene models provide numerous challenges for gene identification and cancer risk assessment. (Cancer Epidemiol Biomarkers Prev 2007;16(3):572–6)

Introduction

There is continued interest in identifying low-penetrance genes for common cancers, and large investments are being made to catalogue the odds ratios (OR) associated with a great number of single-nucleotide polymorphism variants. Some single-nucleotide polymorphisms are chosen for study because they occur in candidate genes, but in other cases, anonymous single-nucleotide polymorphisms (100,000 or more) are studied simultaneously using array-based technology with the intention of representing the entire genome. In each scenario, it is important to have access to a large well-defined population of cancer cases and controls. In Poland, we are developing a series of cancer registries that contain clinical information and DNA specimens from unselected patients with the purpose of identifying the principal genes involved in susceptibility to common forms of cancer. Using this resource, we have recently reported that four Polish founder alleles of the CHEK2 gene predispose to cancers of multiple sites: three of the four alleles are protein truncating (del5395, 1100delC, and IVS2 + 1G>A) and one is a missense variant (I157T) that is present in 5% of Polish controls (1, 2).

It was reported in 2003 by Dong et al. (3) that truncating CHEK2 mutations predispose to cancer of the prostate. We confirmed this association for both the protein-truncating and missense variants (1). The I157T variant has also been found to be associated with prostate cancer risk in Finland (4).

In the course of investigating other candidate prostate cancer genes, we studied genetic variants in the p27 gene (CDKN1B, Kip1). p27 contains a polymorphic variant at position 326 (T/G), which results in the substitution of a valine (V) with a glycine (G) residue. The gene was chosen because, in a previous study, the VV genotype was found to be associated with an increased risk of advanced prostate carcinoma [OR, 2.0; 95% confidence interval (95% CI), 1.1-3.5; ref. 5].

In the present study, we sought to establish whether inherited variation in p27 influences prostate cancer risk, if there is an interactive effect between the presence of the CHEK2 and p27 alleles on prostate cancer risk, and if the relationship between CHEK2 and p27 mutations is specific to prostate cancer.

Materials and Methods

The case groups consisted of 1,519 prostate cancer patients (mean age of diagnosis, 67.4), 872 colon cancer patients (mean age of diagnosis, 63.3), and 1,956 breast cancer patients (mean age of diagnosis, 55.2). Cases were collected from hospitals in Szczecin and in surrounding counties. Study subjects were asked to participate at the time of diagnosis or during an outpatient visit to an oncology clinic. The patient participation rate exceeded 75% for each site. Study subjects were unselected for age and family history. The study was approved by the ethics committee of the Pomeranian Medical University.

Three control groups were combined. The first group consisted of 2,183 newborn children from nine hospitals throughout Poland (Szczecin, Białystok, Gorzow, Katowice, Wroclaw, Poznan, Opole, and Rzeszow) in 2003 and 2006. Samples of cord blood from unselected infants were forwarded to the study center in Szczecin. The second control group consisted of 1,112 young adults from Szczecin who submitted a blood sample for paternity testing. A sample of DNA was forwarded to the reference laboratory without identifying information. The third control group included 517 unselected men above 50 years (mean age, 65 years) and 984 unselected women (mean age, 58.3 years) taken from adult patient rolls of three family doctors practicing in Szczecin. To ensure comparability of the control groups, the allele frequencies of the three alleles were computed separately for the adult and neonatal control groups and compared.

To establish the range of sequence variation of p27 in Poland, the entire 597-bp coding region of the p27 gene was sequenced in 94 unrelated males with familial prostate cancer. The primers were as follows: exon 1, 5'-tcggttttgtttttttgagagtgc-3'; exon 2, 5'-tcggttttgtttttttgagagtgc-3'; and exon 3, 5'-tcggttttgtttttttgagagtgc-3'.

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Table 1. Combined effect of CHEK2-truncating and p27 VV variants on prostate cancer risk, alone and in combination

<table>
<thead>
<tr>
<th>Variants present</th>
<th>Cases (n = 1,519)</th>
<th>Controls (n = 4,796)</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neither variant (CHEK-negative and p27 VG/GG)</td>
<td>476 (56*)</td>
<td>1,630 (173*)</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>CHEK2 truncating alone (CHEK2-positive and p27 VG/GG)</td>
<td>9 (0*)</td>
<td>21 (3*)</td>
<td>1.5 (0.7-3.2)</td>
<td>0.3</td>
</tr>
<tr>
<td>p27 VG alone (CHEK2-negative and p27 VV)</td>
<td>1,004</td>
<td>3,115</td>
<td>1.1 (0.98-1.25)</td>
<td>0.12</td>
</tr>
<tr>
<td>CHEK2 truncating and p27 VV (CHEK2-positive and p27 VV)</td>
<td>30</td>
<td>30</td>
<td>3.4 (2.0-5.7)</td>
<td>0.000003</td>
</tr>
<tr>
<td>Interaction: CHEK truncating × p27 VV</td>
<td></td>
<td></td>
<td>2.1 (0.8-5.4)</td>
<td>0.12</td>
</tr>
</tbody>
</table>

NOTE: The interaction term refers to the extent to which the joint effect of having both alleles is greater than expected. CHEK2-truncating variants include IVS2 + 1G>A (15 cases/20 controls) or IVS2delEC (13 cases/11 controls) or delE5395 (11 cases/20 controls).

*Contribution of GG genotype among VG or GG genotype carriers.

(p27e1F) and 5′-ggccccacaaccactcttg (p27e1R) and exon 2, 5′-cacaattctgcacgcatgt (p27e2F) and 5′-taagatcaggtatcgtgaggtc (p27e2R). Only two sequence variants were detected: 31C>T (Pro11Ser) and 326T>G (V109G).

We assessed the frequency of the two p27 variants in a larger series of cases and controls by means of RFLP PCR assay. The 31C>T variant was analyzed using primers p27e1F and p27e1R and AluI enzyme. The PCR product was digested when the T allele was present. The 326T>G allele was detected with the same primers and BglII digestion. The PCR product was digested in samples where the G allele was present. Other naturally occurring restriction site for AluI and BglII enzymes within exon 1 served as internal control in both assays, which confirmed complete digestion in all samples. The assays were also validated by RFLP analysis of the cases that were previously sequenced, and RFLP showed the same results as sequencing.

We evaluated the presence of four CHEK2 variants in the cases and controls, including one missense variant (I157T), two truncating variants (1100delC and IVS2 + 1G>A), and one deletion (delE5395). The techniques for detection of these mutations have been described previously (2, 6).

Statistical analysis included the comparison of the proportions of the prevalence of the allele in cases and controls. ORs were generated from two-by-two tables, and statistical significance was assessed using the Fisher’s exact test. The means were compared using unpaired t test. To evaluate the statistical significance of the observed gene-gene interaction, a multivariable unconditional logistic regression was conducted using a model that contained terms for the main effects of the CHEK2 and p27 variants as well as an interaction term.

Results

To establish the range of sequence variation of p27 in Poland, we sequenced the entire 597-bp coding region of p27 gene in 94 unrelated males with familial prostate cancer. Two sequence variants were detected within the p27 gene: 31C>T (Pro11Ser) and 326T>G (V109G). We assessed the frequency of the two p27 variants in larger series of cases and controls. We found the 31C>T (Pro11Ser) in only 1 of 419 analyzed prostate cancer cases and in none of 514 controls (adult men above the age of 50 years), and it was not studied further. The 326T>G (V109G) variant was more common: the homozygous genotype 326 TT (V109V) was present in 68.1% of 1,519 prostate cancer cases compared with 65.6% of the 4,796 controls (OR, 1.1; P = 0.08).

A truncating CHEK2 variant was present in 2.6% of the prostate cancer cases and in 1.1% of the controls (OR, 2.5; P < 0.0001). The CHEK2 missense variant I157T was present in 7.8% of 1,519 prostate cancer cases and in 4.9% of the controls (OR, 1.6; P < 0.0001).

Because we were interested in estimating the extent of risk associated with common population variants in combination, a two-gene analysis was done (Table 1). In this analysis, the effect of each variant allele, alone and in combination, was measured by comparison with a referent group of men with neither variant. Among men with the p27 VG/GG background, the OR for prostate cancer, given a truncating CHEK2 mutation, was 1.5 (P = 0.3). In contrast, among men with the p27 VV genotype, the OR associated with a truncating CHEK2 mutation was 3.1 (P < 0.0001). A formal test of interaction was conducted using unconditional logistic regression. The interaction, however, did not reach statistical significance (P = 0.1). For men with both variants (compared with neither), the OR was 3.4 (P < 0.0001). The VV p27 genotype was overrepresented in men with prostate cancer and a truncating CHEK2 mutation: 77% of 39 men with prostate cancer and a CHEK2 truncating mutation of either type carried the VV background compared with 59% of 51 controls (P = 0.08).

We then studied this possible interaction in men who carried the I157T mutation in CHEK2 (Table 2). Among men with the p27 VG/GG background, the I157T allele of CHEK2 did not significantly elevate the risk of prostate cancer (OR, 1.3; P = 0.3). In contrast, on the VV background, the CHEK2 I157T allele was associated with a highly significant increase in cancer risk (OR, 1.9; P < 0.0001). Similarly, the p27 allele in isolation was not associated with a significant increase in risk (OR, 1.1; P = 0.2). The combined genotype was found to be associated with a highly significant 2-fold increase in risk of prostate cancer (P = 0.000001), although again the interaction term was not statistically significant (P = 0.1). The VV p27 genotype was overrepresented in men with prostate cancer and a missense CHEK2 mutation: 72% of the men with prostate cancer and I157T mutation carried the VV background compared with 61% of healthy controls with this CHEK2 mutation (P = 0.05). Thus, in a second independent set of CHEK2 mutation carriers, we confirmed that the OR associated with a CHEK2 was greater when on the VV p27 genetic

Table 2. Combined effect of CHEK2-missense (I157T) and p27 VV variants on prostate cancer risk, alone and in combination

<table>
<thead>
<tr>
<th>Variants present</th>
<th>Cases (n = 1,519)</th>
<th>Controls (n = 4,796)</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neither variant (CHEK-negative and p27 VG/GG)</td>
<td>452 (53*)</td>
<td>1,560 (165*)</td>
<td>1.0</td>
<td>—</td>
</tr>
<tr>
<td>CHEK2 I157T alone (CHEK2-positive and p27 VG/GG)</td>
<td>33 (3*)</td>
<td>91 (11*)</td>
<td>1.3 (0.8-1.9)</td>
<td>0.3</td>
</tr>
<tr>
<td>p27VV alone (CHEK2-negative and p27 VV)</td>
<td>948</td>
<td>3,000</td>
<td>1.1 (1.0-1.2)</td>
<td>0.2</td>
</tr>
<tr>
<td>CHEK2 I157T and p27 VV (CHEK2-positive and p27 VV)</td>
<td>86</td>
<td>145</td>
<td>2.0 (1.5-2.7)</td>
<td>0.000001</td>
</tr>
<tr>
<td>Interaction: CHEK2 missense × p27 VV</td>
<td></td>
<td></td>
<td>1.5 (0.9-2.5)</td>
<td>0.11</td>
</tr>
</tbody>
</table>

NOTE: The interaction term refers to the extent to which the joint effect of having both alleles is greater than expected.

*Contribution of GG genotype among VG or GG genotype carriers.
background. Overall, 73% of 157 men with prostate cancer and a CHEK2 mutation of either type carried the VV background compared with 61% of 286 controls (P = 0.009).

We next sought to establish if the relationship between CHEK2 and p27 mutations was prostate specific or was a more general phenomenon. We previously reported a modest association between the CHEK2 I157T allele and colon cancer risk on a series of 300 cases with colon cancer (1). In our larger current series (872 cases), the CHEK2 missense variant I157T was present in 7.1% of colon cancer cases compared with 4.9% of the controls (OR, 1.5; P = 0.01). A truncating variant was present in 0.9% of cases and 1.1% of controls (OR, 0.9; P = 0.8). These cases were now genotyped for the p27 polymorphism. A small but significant effect was found for the p27 allele on colon cancer risk (OR, 1.3; 95% CI, 1.1-1.5; P = 0.003). Again, there was evidence for an interactive effect between the presence of the CHEK2 and p27 alleles (because of the small number of truncating variants, the two categories of mutations were combined; Table 3). On the VG/GG p27 background, the CHEK2 allele was not associated with an increased risk of colon cancer (OR, 0.8; P = 0.5), but on the VV background, the OR for colon cancer, given a CHEK2 mutation, was 1.7 (P = 0.002). In this case, test for interaction was statistically significant (P = 0.04).

We also genotyped 1,965 cases of breast cancer. Main effects were seen both for truncating CHEK2 mutations (OR, 2.4; P < 0.0001) and for missense mutations (OR, 1.4; P = 0.004). There was no effect of p27 on breast cancer risk (OR, 0.9; 95% CI, 0.8-1.0), and there was no observed interaction with p27 (Table 4).

In the previous analyses, we considered the VV genotype of the p27 gene to be the genotype of risk, and we categorized patients as those with the VV genotype and those with either the VG or GG genotypes. We have also conducted similar analyses comparing the effects of the VG and GG genotypes separately. These analyses did not reveal any increased risks associated with the VG genotype, and therefore, individuals with the VG and GG genotypes were combined into a single group.

In summary, in patients with prostate cancer and colon cancer (but not with breast cancer), we found the increased risk of cancer associated with a mutant CHEK2 allele to be greater among patients who were homozygous for the VV genotype at the p27 locus.

### Discussion

Aberrant cell cycle control is a hallmark of cancer (7). In eukaryotes, the cell cycle is driven by cyclin-dependent kinases (CDK) and negatively regulated by CDK inhibitors (8). Alterations in CDK inhibitor expression have been implicated in malignancy in general and in prostate carcinoma in particular (9-12). p27 is a member of CDK inhibitors Cip/Kip family and is involved in the cell cycle control. p27 inhibits activation of CDK2-cyclin E complexes, preventing cell from progression to the S phase of the cell cycle (causing G1/S cell arrest). CHEK2 is one of the genes in the DNA damage signaling pathway. CHEK2, activated by DNA damage, phosphorylates Cdc25A phosphatase, which results in inhibition of CDK2-cyclin E complexes and G1/S cell arrest. In addition, CHEK2 inhibits cyclin D, and thereby maintaining G1-S arrest (13).

Thus, a possible explanation for the epistatic interaction between the two genes in the different pathways is that both p27 and CHEK2 finally regulate the G1-S checkpoint. In this model, mutation of both genes might inhibit cell cycle arrest more effectively than the mutation in only one of these.

Another possible explanation is that loss of p27 function promotes overexpression of cyclin E or cyclin D, and this overexpression lengthens S phase and facilitates DNA damage (14), which is then not effectively repaired when CHEK2 is mutated.

The p27 V109G polymorphism lies within the p38Rab1 binding domain. p38Rab1 functions as a negative regulator of p27 by promoting degradation. The absence of mutations, coupled with multiple studies showing decreased p27 protein expression in malignancy, has led to speculation that decreased p27 levels in malignancy are secondary to alterations in degradation. It is possible that the V allele may alter p27 affinity for p38Rab1 and thereby modify CDKN1B degradation (15).

Our principal finding was that the effect of the two susceptibility alleles in combination exceeded the expected risk based on a purely multiplicative model. The differences in risk were relatively large, and the magnitude of the interaction terms was 2.1 and 1.5 for prostate cancer (truncating and

<table>
<thead>
<tr>
<th>Variants present</th>
<th>Cases (n = 1,965)</th>
<th>Controls (n = 4,796)</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neither variant (CHEK-negative and p27 VG/GG)</td>
<td>642 (80%)</td>
<td>1,539 (162%)</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>CHEK2 mutation alone (CHEK2-positive and p27 VG/GG)</td>
<td>74 (8%)</td>
<td>112 (14%)</td>
<td>1.6 (1.2-2.2)</td>
<td>0.003</td>
</tr>
<tr>
<td>p27 VV alone (CHEK2-negative and p27 VV)</td>
<td>1,138</td>
<td>2971</td>
<td>0.9 (0.8-1.0)</td>
<td>0.1</td>
</tr>
<tr>
<td>CHEK2 mutation and p27 VV (CHEK2-positive and p27 VV)</td>
<td>111</td>
<td>174</td>
<td>1.5 (1.2-2.0)</td>
<td>0.001</td>
</tr>
<tr>
<td>Interaction: CHEK2 × p27 VV</td>
<td></td>
<td></td>
<td>1.1 (0.7-1.6)</td>
<td>0.8</td>
</tr>
</tbody>
</table>

NOTE: The interaction term refers to the extent to which the joint effect of having both alleles is greater than expected. CHEK2 mutations include I157T (135 cases/236 controls), IVS2 +1G>A (21 cases/20 controls), 1100delC (10 cases/11 controls), or del5395 (19 cases/20 controls).

*Contribution of GG genotype among VG or GG genotype carriers.
missense variants, respectively) and 2.0 for colon cancer (all CHEK2 mutations), although only the last term was statistically significant (P = 0.04). Recently, it was reported that p27 and PTEN interact epitaxically in a mouse prostate cancer model. Mice who were heterozygous knockouts for both genes experienced a 100% lifetime risk of prostate cancer (16). In a follow-up study, Xu et al. (17) found a greater than expected degree of allelic sharing in affected members of prostate cancer families at the chromosomal loci containing the human homologues of these two genes. We did not identify any common PTEN polymorphisms in the Polish population.

An initial study by Kibel et al. (5) found that the risk conferred by the p27 VV genotype was higher in earlier-onset prostate cancer patients. We saw no clear association between the presence of p27 VV or CHEK2 variants and age at onset for any of the studied cancers. The mean ages of diagnosis in p27 VV-positive and p27 VV-negative cases, in CHEK mutation-positive and CHEK mutation-negative cases, and in cases with and without the combined CHEK2/p27 genotype were within ±1.5 years from the mean age at diagnosis in a series of unselected cases for each site (all P values for difference were >0.1).

We studied 4,796 controls to generate precise estimates of the frequencies of the different alleles in the underlying population. All controls were ethnic Poles, but we included both newborn and adult controls and both males and females. Our choice of controls is based on the premise that the allele frequencies in the Polish population do not vary with age or sex; this was confirmed in subgroup analyses (Table 5).

There are interesting consequences of the two-gene epistatic model, which are consistent with some unusual epidemiologic observations. The CHEK2 1100delC mutation does not clearly cosegregate with the cancer phenotype in multiply-affected families. Because of this, several authors have suggested that CHEK2 might act in synergy with other cancer susceptibility genes (18-20). We propose that p27 is the first such modifying gene to be identified; there might be others as well. In this scenario, if a relative does not carry the susceptibility genotype at the second (modifying) locus, then the cancer risk associated with the CHEK2 mutation would not be elevated, and we would expect to see poor segregation between the CHEK2 allele and the appearance of cancer in other family members. It will be difficult to identify the main effects of an epistatic cancer susceptibility gene using a linkage approach if a single-gene dominant model is assumed. It is noteworthy in this regard that recent efforts in linkage analyses of prostate cancer and breast cancer (post-BRCA2) have been largely unsuccessful.

Furthermore, we might expect to see a discrepancy between the relative risk of first primary and second primary (or contralateral) cancer (the relative risk for second cancers would be higher than the relative risk for first-degree relatives). This is because the population of patients with cancer and a CHEK2 mutation is more likely to carry the epistatic allele than the population of their relatives. In our study, 73% of the prostate cancer patients and 80% of the colon cancer patients were carriers of the p27 VV background genotype versus 61% of unselected controls.

Under the epistatic model, cancer risk would best be evaluated considering the joint genotypes at two loci. In our population, the main effects of each of the two genes on prostate and colon cancer risk were modest (range, 1.1-2.6). The effects in combination were more substantial (range, 2.0-3.4). However, if it were necessary to search for all possible two-gene combinations following a genomic search, the number of possibilities would be enormous. For example, if a 100K single-nucleotide polymorphism array were used, there would be 5 × 10^8 possible biallelic combinations to interrogate, a number greatly exceeding the total number of cancers diagnosed annually worldwide.

We propose that the clinical expression of CHEK2 mutations on prostate and colon cancer risk may depend on the genetic background, and the p27 locus is the first such modifying gene for CHEK2 to be identified. Our observations have potentially great implications for the field of cancer genetics. In theory, the risk associated with truncating CHEK2 mutations might vary from 0.8 to 3.1 based on the population frequency of the V allele of p27. It is important that these observations be replicated in other centers. It is interesting to speculate that there might be other genes that interact with CHEK2 as well.

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References


Table 5. Frequencies of p27 and CHEK2 variants in adult and newborn controls

<table>
<thead>
<tr>
<th>Control group</th>
<th>Variant no./frequency</th>
<th>Genotype no./frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p27 VV, n (%)</td>
<td>CHEK2 I157T, n (%)</td>
</tr>
<tr>
<td>Newborns (n = 2,183)</td>
<td>1,419 (65.0)</td>
<td>101 (4.6)</td>
</tr>
<tr>
<td>Young adults (paternity testing; n = 1,112)</td>
<td>729 (65.5)</td>
<td>56 (5.0)</td>
</tr>
<tr>
<td>Elderly adults (family doctors; n = 1,501)</td>
<td>997 (66.4)</td>
<td>79 (5.3)</td>
</tr>
<tr>
<td>Adult men (n = 1,082)</td>
<td>708 (65.4)</td>
<td>54 (5.0)</td>
</tr>
<tr>
<td>Adult women (n = 1,531)</td>
<td>1,018 (66.5)</td>
<td>81 (5.3)</td>
</tr>
</tbody>
</table>

P < 0.01; n = number of individuals; % = percentage of population; n = number of individuals; % = percentage of population.


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