Constitutional CHEK2 mutations are associated with a decreased risk of lung and laryngeal cancers

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Mutations in the CHEK2 gene have been associated with increased risks of breast, prostate and colon cancer. In contrast, a previous report suggests that individuals with the 1157T missense variant of the CHEK2 gene might be at decreased risk of lung cancer and upper aero-digestive cancers. To confirm this hypothesis, we genotyped 895 cases of lung cancer, 430 cases of laryngeal cancer and 6391 controls from Poland for four founder alleles in the CHEK2 gene, each of which has been associated with an increased risk of cancer at several sites. The presence of a CHEK2 mutation was protective against both lung cancer (odds ratio (OR) = 0.3; 95% confidence interval (CI) 0.2–0.5) and laryngeal cancer (OR = 0.6; 95% CI 0.3–0.99; P = 0.05). The basis of the protective effect is unknown, but may relate to the reduced viability of lung cancer cells with a CHEK2 mutation. Lung cancers frequently possess other defects in genes in the DNA damage response pathway (e.g. p53 mutations) and have a high level of genotoxic DNA damage induced by tobacco smoke. We speculate that lung cancer cells with impaired CHEK2 function undergo increased rates of cell death.

Introduction

Germ line mutations in CHEK2 have been associated with a range of cancer types, in particular of the breast and the prostate, but cancers of the kidney, thyroid and colon have also been implicated (1–12). In Poland, there are four known founder alleles of CHEK2, three alleles are protein truncating and the other is a missense variant (1157T). All four alleles have been associated with an increased risk of cancer (5,13). For breast and prostate cancers, the observed associations are stronger for the protein-truncating alleles than for the missense allele (13,14), but for colon cancer, only the missense allele appears to be pathogenic (15,16). It appears therefore that the risk of cancer associated with CHEK2 mutations varies, depending on the cancer site and the class of mutation studied.

In our 2004 study (5), we observed a low frequency of CHEK2 mutations in a sample of 272 unselected lung cancer patients, compared with 4000 controls in Poland (2.6% versus 5.5%). Following our initial results, Brennan et al. (17) found a highly significant protective effect of the 1157T missense variant, both for lung cancer and for upper aero-digestive cancers, in a large case-control study conducted in Central and Eastern Europe. We wished to extend our initial findings and those of Brennan et al. We have extended our series of lung cancer cases from 272 to 895 and our control sample from 4000 to 6391. We have also identified a fourth deleterious CHEK2 allele (a large deletion of exons 9 and 10). Because smoking is the principal risk factor for lung cancer in Poland and elsewhere, we asked whether the protective effect of CHEK2 might extend to laryngeal cancer patients as well.

Materials and methods

We studied 895 unselected cases of lung cancer (226 women and 669 men) diagnosed in the Lung Diseases Hospital in Szczecin, Poland, between 2004 and 2006. We also ascertained 430 consecutive, unselected patients with squamous cell carcinoma of the larynx (70 women and 360 men) at Department of Otolaryngology and Laryngological Oncology of the Pomeranian Medical University, Szczecin, Poland, during the period 2001–2004. Patients were recruited from the oncology services of the contributing hospitals and were unselected for age or family history. Patients were approached by a member of the study team during an outpatient visit to the oncology clinic and were asked if they wished to participate. Patient acceptance rates exceeded 80% for both cancer sites. Patients provided written informed consent. A blood sample of 10 cc was then drawn for DNA extraction. Two hundred and seventy-two of the lung cancer patients have been included in our previous study (5). The mean age of diagnosis of the lung cancer patients was 61.4 years (range 29–88 years) and of the laryngeal cancer patients was 58.2 years (range 30–84). Patients completed a questionnaire about their smoking habits at the time of cancer diagnosis. Smoking histories were available for 818 of 895 (91%) lung cancer cases and for 387 of 430 (90%) laryngeal cancer cases. The study was approved by the Ethics Committee of the Pomeranian Medical University in Szczecin.

Unmatched analysis

In the unmatched analysis, four non-overlapping control groups were combined in order to maximize the number of controls. The first control group consisted of 1896 healthy adults, including 1079 women (age range 15–91, mean 58.3) and 817 men (age range 23–90, mean 59.4). These controls were selected at random from the computerized patient lists of five large family practices located in the region of Szczecin. These healthy adults were invited to participate by mail and participated in 2003 and 2004. Participation rates for this group exceeded 70%. During the interview, the goals of the study were explained, informed consent was obtained, genetic counselling was given and a blood sample was taken for DNA analysis. A detailed family history of cancer was taken (first- and second-degree relatives included). Prospects were included regardless of their cancer family history status. Individuals affected with any malignancy were excluded from the study.

The second control group consisted of 1417 unselected young adults (705 women and 712 men; age range 18–35, mean 24.3) from Szczecin metropolitan region who submitted a blood sample for paternity testing between 1994 and 2001.

The third control group consisted of 2183 children from nine cities in Poland (Szczecin, Białystok, Gorzów, Katowice, Wrocław, Poznań, Opol, Łódź and Rzeszów) between 2003 and 2006. Seven hundred and ninety-nine infants were from Szczecin and 1384 were from other cities. Samples of cord blood from unselected infants were forwarded to the study centre in Szczecin.

The fourth group of control subjects consisted of 895 healthy adults (226 women and 669 men) with a negative family history of cancer from the region of Szczecin. These individuals were chosen for this study to be sex-matched, age-matched and geographically matched with the lung cancer cases. Age was matched within 2 years. The healthy adults were part of a population-based study of the 1.5 million residents of West Pomerania designed to identify familial aggregations of cancer by our centre. These subjects were invited for an interview in 2007. During the interview, the goals of the study were explained, informed consent was obtained and a blood sample was taken for DNA analysis. A detailed family history of cancer was taken (first- and second-degree relatives included) and a risk factor questionnaire was completed, including smoking history. Individuals affected with any malignancy or with any cancers diagnosed in a first-degree relative were excluded from this control group.

In total, there were 6391 controls. The allele frequencies for the various CHEK2 variants were similar in the younger and older individuals, and in males and females (shown in Table I). Also there was no observed difference between the allele frequencies among infants from Szczecin metropolitan area.

Abbreviations: CI, confidence interval; OR, odds ratio; PCR, polymerase chain reaction.
region and from other cities; therefore, the four control groups were combined to
generate the most precise estimates of the frequencies of the four CHEK2
mutations in the underlying Polish population.

Matched analysis
A matched analysis was also performed in order to investigate whether or not
tobacco exposure is an important modifier of the possible association between
CHEK2 mutations and lung and laryngeal cancer. In this analysis, two matched
controls were selected for each case, selected on sex and year of birth. Age-
and sex-matched controls were selected from the control set described above.
We were able to identify 1692 suitable matched controls for the lung cancer
cases. Smoking status was available for 1594 of these controls (94%). We also
defined 860 age- and sex-matched controls for the laryngeal cancer cases (Table II).

Genotyping
DNA was isolated from 5 to 10 ml peripheral blood. Two primers pairs were
used for genotyping of the large deletion of exons 9 and 10 in a multiplex
polymerase chain reaction (PCR) as described previously (13). The first primer
pair flanked the breakpoint site in intron 8 and the second primer pair flanked
the breakpoint site in intron 10. In mutation-negative cases, only two PCR
fragments of 379 and 522 bp were amplified from the wild-type allele. In
mutation-positive cases, the forward primer of the first primer and the reverse
primer of the second primer amplified an additional PCR product of 450 bp.
The other three mutations in CHEK2 (IVS2 þ 1G> A, 1100delC and I157T)
were genotyped as described previously (5). These variants are detected by
Allele specific oligonucleotide or Restriction fragment length polymorphism–
PCR analyses. In all reaction sets, positive and negative controls (without
dNA) were used. All PCRs or enzymatic digestions were performed under a
layer of mineral oil. Duplicate genotyping for quality control was performed for
462 randomly selected individuals, but no discrepancies with the initial
results were found. As a further check, all mutation-positive cases were con-
irmed by sequencing, but again with no discrepancies.

There is evidence that all four CHEK2 alleles present in Poland are in the
functional copy of CHEK2 gene at chromosome 22 (not in a pseudogene
located elsewhere) (5,13,18).

Statistical analysis
The prevalence of each of the four CHEK2 alleles was compared in cases and
in controls, singly and in combination. The three protein-truncating mutations
were studied separately from the missense variant. Odds ratios (ORs) were
generated from two-by-two tables and statistical significance was assessed
using the Fisher exact test for the unmatched analysis. The ORs were generated
separately for lung and laryngeal cancer patients. The analysis was con-
ducted separately for the unmatched and matched analyses, for smokers and
non-smokers.

Results
In total, 1325 cancer cases and 6391 controls were genotyped for the
four CHEK2 variants. A CHEK2 mutation was found in 1.8% of the
lung cancer cases, in 3.5% of the laryngeal cancer cases and in 5.8%
of the controls. Both the truncating variants and the missense var-
iants were observed more frequently in the controls than in the cases (Table III).

<table>
<thead>
<tr>
<th>Table I. Frequencies of CHEK2 variants in controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Young controls (paternity testing)</td>
</tr>
<tr>
<td>Older controls (family doctors)</td>
</tr>
<tr>
<td>‘Matched’ adults</td>
</tr>
<tr>
<td>Newborns</td>
</tr>
<tr>
<td>All controls</td>
</tr>
</tbody>
</table>

*Any truncating mutation—del5395 or IVS2 þ 1G> A or 1100delC.

Two controls were homozygous for the I157T variant. One controls had
a truncating mutation and the I157T variant.

A strong protective effect against lung cancer was seen for truncat-
ing and missense mutations combined [OR = 0.3; 95% confidence
interval (CI) 0.2–0.5; P = 3 × 10–8]. The mean age of onset of lung
cancer in carriers of a CHEK2 mutation was 3.5 years older than in
non-carriers (65.1 years versus 61.6 years, respectively) but the dif-
ference was not significant (P = 0.1). Results by histologic subtype
are given in Table IV. A very strong protective effect was observed for
squamous cell cancer (OR = 0.2; 95% CI 0.1–0.5). Protective effects
were also seen for small cell and adenoscarcinoma subtypes, but these
were not statistically significant. A stronger protective effect for lung
cancer was seen for men (OR = 0.2; 95% CI 0.1–0.5) than for women
(OR = 0.4; 95% CI 0.2–1.0). A modest effect of a CHEK2 mutation (all variants combined) was
also seen for squamous cell carcinoma of the larynx (OR = 0.6; 95% CI
0.3–0.99; P = 0.05). Again, a stronger effect was seen for men
(OR = 0.5; 95% CI 0.3–1.0) than for women (OR = 0.7; 95% CI
0.2–2.3). The mean ages of onset of laryngeal cancer in carriers and
non-carriers were similar (56.1 years versus 58.3 years, respectively;
P = 0.3).

In order to ensure that the observed effect was not due to incompati-
ble of the cases and controls, a matched analysis was per-
formed. Each case was matched to two controls for age, sex and
geographical region (Table II). In this analysis, a protective effect of a similar size was seen for lung cancer (OR = 0.3; P = 0.000002) and laryngeal cancer (OR = 0.6; P = 0.06) (Table V).

<table>
<thead>
<tr>
<th>Table II. Comparison of cases and controls: matched analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
</tr>
<tr>
<td>-------------------------------------------------------------</td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td>Mean (range)</td>
</tr>
<tr>
<td>Sex</td>
</tr>
<tr>
<td>Males, n (%)</td>
</tr>
<tr>
<td>Females, n (%)</td>
</tr>
<tr>
<td>Smoking</td>
</tr>
<tr>
<td>Ever, n (%)</td>
</tr>
</tbody>
</table>

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CHEK2 is a multiorgan tumour susceptibility gene (5,19). Interestingly, both p53 somatic inactivation and a high level of DNA damage induced by smoking exposure are characteristics of lung cancer (20). The frequency of somatic mutations in p53 in lung cancer is higher (~70% in smokers) than in breast or prostate cancers (~20%), and the p53 mutational patterns are different between these cancers (21–23). These differences may in part explain the different effects of CHEK2 mutation on lung, breast and prostate cancer risks.

It has been suggested that CHK2 inhibitors might be useful in cancer treatment, and the success to this type of therapy is likely to depend on the mutational profile of the targeted cancer cell, in particular its p53 status (24). The expression of normal CHK2 protein

Table IV. Effect of CHEK2 truncating and missense mutation on lung cancer risk, by histologic subtype

<table>
<thead>
<tr>
<th>Histological type</th>
<th>Truncating</th>
<th>Missense</th>
<th>Total OR 95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squamous, ( n = 352 )</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>0.2</td>
</tr>
<tr>
<td>Small cell, ( n = 111 )</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>0.3</td>
</tr>
<tr>
<td>Adenocarcinoma, ( n = 182 )</td>
<td>0</td>
<td>5</td>
<td>5</td>
<td>0.5</td>
</tr>
<tr>
<td>Other or unclassified, ( n = 250 )</td>
<td>1</td>
<td>4</td>
<td>5</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Table V. Effect of CHEK2 mutations on lung and laryngeal cancer risk; matched analysis

<table>
<thead>
<tr>
<th></th>
<th>Cases, ( n )</th>
<th>CHEK2 positive, ( n \ (% )</th>
<th>Controls ( n )</th>
<th>CHEK2 positive, ( n \ (% )</th>
<th>OR 95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>895</td>
<td>16 (1.8%)</td>
<td>1692</td>
<td>95 (5.6%)</td>
<td>0.3</td>
<td>0.2–0.5</td>
</tr>
<tr>
<td>Non-smokers</td>
<td>49</td>
<td>2 (4.1%)</td>
<td>615</td>
<td>38 (6.2%)</td>
<td>0.6</td>
<td>0.2–2.8</td>
</tr>
<tr>
<td>Smokers</td>
<td>769</td>
<td>14 (1.8%)</td>
<td>979</td>
<td>53 (5.4%)</td>
<td>0.3</td>
<td>0.2–0.6</td>
</tr>
<tr>
<td>Pack years (&lt;20)</td>
<td>91</td>
<td>1 (1.2%)</td>
<td>390</td>
<td>22 (5.6%)</td>
<td>0.2</td>
<td>0.02–1.4</td>
</tr>
<tr>
<td>Pack years (\geq20)</td>
<td>678</td>
<td>13 (2.7%)</td>
<td>589</td>
<td>33 (5.6%)</td>
<td>0.3</td>
<td>0.2–0.6</td>
</tr>
<tr>
<td>Laryngeal cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>430</td>
<td>15 (3.5%)</td>
<td>860</td>
<td>51 (5.9%)</td>
<td>0.6</td>
<td>1.3–1.03</td>
</tr>
<tr>
<td>Non-smokers</td>
<td>15</td>
<td>1 (6.7%)</td>
<td>310</td>
<td>20 (6.4%)</td>
<td>1.0</td>
<td>0.1–8.3</td>
</tr>
<tr>
<td>Smokers</td>
<td>372</td>
<td>13 (3.5%)</td>
<td>550</td>
<td>31 (5.6%)</td>
<td>0.6</td>
<td>0.3–1.2</td>
</tr>
<tr>
<td>Pack years (&lt;20)</td>
<td>72</td>
<td>2 (2.8%)</td>
<td>203</td>
<td>11 (5.4%)</td>
<td>0.5</td>
<td>0.1–2.3</td>
</tr>
<tr>
<td>Pack years (\geq20)</td>
<td>300</td>
<td>12 (4.0%)</td>
<td>347</td>
<td>20 (5.8%)</td>
<td>0.7</td>
<td>0.3–1.4</td>
</tr>
</tbody>
</table>

Discussion

We have shown that the presence of an inherited CHEK2 mutation is associated with a decrease in the risk of two of the principal smoking-related cancers in Poland. These results are in agreement with those of Brennan et al. (17) who also investigated cases of Eastern European origin, but restricted the study to a single CHEK21157T mutation. In our study, both truncating and missense CHEK2 mutations appear to be protective against lung and laryngeal cancer. The ORs given the I157T in the two studies (this study, (17)) are remarkably similar and all are highly significant. In both studies, the strongest protective effect is seen for squamous subtype of lung cancer (OR = 0.2). Our paper also suggests a protective effect of three CHEK2 truncating alleles on lung and laryngeal cancer risks. These results contrast with our observations that both truncating and missense alleles are associated with increased risks of prostate and breast cancers in Poland (13,14).

The strengths of our study include large number of cases and controls, and the sampling of incident cases, unselected for age or family history. Cases and controls are all ethnic Poles. There is no reason to believe that our findings could be the result of inappropriate selection of controls. Allele frequencies were similar in controls of different ages and sex (Table I). The frequency of I157T in our controls (4.8%) is similar to that reported later by Brennan et al. (17) in a non-overlapping series of 790 controls from Poland (5.6%). The frequency of the I1100delC in both series is almost identical (0.2%). Using the same control population, we previously reported that CHEK2 mutations increase the risk of cancer in several different sites including the breast, prostate, thyroid, colon and kidney (5). Furthermore, in a subanalysis in which the cases and controls were matched for age and sex, the ORs were almost identical to those of the larger, unmatched analysis.

CHK2 is a versatile and multifunctional kinase that regulates the cellular response to DNA damage by phosphorylating a number of cellular substrates. As such, it might prevent tumour progression by averting genomic instability through DNA repair or by causing the cell to senesce or to die. CHK2 regulates DNA repair through phosphorylation of BRCA1, which functions within the error-free homologous recombination repair pathway. CHK2 is implicated in activating p53-mediated cell-cycle arrest and apoptosis. CHK2 can also promote apoptosis through a p53-independent mechanism. CHK2 is required for the release of survivin from mitochondria, which is thought to inhibit apoptosis in cancer cells. This interpretation of CHK2 function correlates with several human studies, which show that CHEK2 is a multiorgan tumour susceptibility gene (5,19).

It is of interest that CHEK2 mutations appear to increase the risk of cancer at some sites (e.g. breast, prostate) and protect against others (lung, larynx). This may relate to the smoking-dependent etiology of lung and laryngeal cancer and the typical mutational profile of these cancer cells. Several lines of evidence suggest that pharmacological inhibition of CHK2 in combination with DNA-damaging agents (ionizing radiation and chemotherapeutics) might trigger cell death in tumours that already possess defects in other genes in the DNA-damage response, in particular p53 (19). By analogy, germ line mutations in CHEK2 might also promote the death of cancer cells which are p53 deficient and which are exposed to a high level of DNA damage. Interestingly, both p53 somatic inactivation and a high level of DNA damage induced by smoking exposure are characteristics of lung cancer (20). The frequency of somatic mutations in p53 in lung cancer is higher (~70% in smokers) than in breast or prostate cancers (~20%), and the p53 mutational patterns are different between these cancers (21–23). These differences may in part explain the different effects of CHEK2 mutation on lung, breast and prostate cancer risks.
may be essential to the viability of lung cancer cells. In particular, CHK2 might be a target of interest in the treatment of patients with lung cancer. It is also possible that the protective effect of mutant CHEK2 alleles is related to a function of CHEK2 that is not yet known. Studies of the role of CHEK2 in lung cancer cells could clarify this.

Acknowledgements

Conflict of Interest Statement: None declared.

References


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