CDKN2A common variant and multi-organ cancer risk—a population-based study

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The population frequencies of the CDKN2A common variants remain undetermined. In Poland, there is a common variant of the CDKN2A: an alanine to threonine substitution (A148T), which has been detected in other populations. We have recently showed that it is significantly overrepresented among Polish melanoma patients when compared to general population. Herein, we ascertained the prevalence of the A148T variant in 3,583 unselected cancer cases and 3,000 random control subjects from the same Polish population. We evaluated eleven different malignancies, representing the majority of all common cancer sites. Positive association with A148T variant was observed for lung cancer (OR, 2.0; p = 0.0052). A similar trend, although nonsignificant after the Bonferroni correction, was observed for colorectal cancer (OR, 1.5; p = 0.5499). These results suggest that A148T variant may be associated with a multi-organ cancer risk in the Polish population.

Patients and methods

Cases were enrolled in the study from hospitals in Szczecin and surrounding counties. Study subjects were asked to participate at the time of diagnosis or during outpatient visits to their surgical and medical oncology clinics. In general, patient participation rates exceeded 75% for each cancer site. Study subjects were unselected for age, sex or family history. Two control groups were combined. The first group consisted of 2,000 newborn children from ten hospitals throughout Poland (Szczecin, Bialystok, Gorzow, Katowice, Wroclaw, Poznan, Opole, Lodz and Rzeszow) in 2003 and 2004. Samples of cord blood from unselected infants were forwarded to the study center in Szczecin. The second control group consisted of 1,000 unselected adults from the region of Szczecin.

To ensure comparability of the control groups, the A148T allele frequencies were computed separately for the adult and neonatal control groups and compared. DNA samples were obtained from peripheral blood of individuals or from umbilical cord blood of newborns. The A148T variant was analyzed by restriction fragment length polymorphism PCR, using np16ex2f (AGGGGTAA-TTAGACACCTGG) and np16ex2r (TTTGGAAGCTCTCAGGG-TAC) primers. PCR products were digested with the SacII enzyme and separated in 2–3% agarose gels. The presence of the A148T change was confirmed by direct DNA sequencing.

Statistical analysis included comparison of the prevalence of the A148T allele in cases and controls. Odds ratios were generated from two-by-two tables and statistical significance was assessed using a chi-square test with Bonferroni correction. Possible deviation of the genotype frequencies from those expected under Hardy-Weinberg equilibrium (HWE) was assessed by the chi-square probability test.

Results

The A148T variant was detected in 3.5% of Polish controls. Our control group was drawn both from the newborns of ten Polish cities and from adult population from the region of Szczecin.
However, the frequency of the alleles was similar in the newborn population (3.4%) compared to the adult population (3.55%) \( (p = 0.8331, 95\% \text{ confidence interval } 0.6308–1.450) \). There was no statistical difference in the CDKN2A allele frequencies in the newborns recruited from the Szczecin metropolitan region compared to other Polish cities.

There was no evidence that the genotype frequencies of the A148T variant deviated from those expected under HWE for the control groups \( (p > 0.4) \). The prevalence of the A148T variant was higher in cancer cases than in controls for three of the eleven sites studied (Table I). The highest odds ratios were observed among lung cancer \( (OR = 2.0) \) and colon cancer \( (OR = 1.5) \) cases. For lung the excess was statistically significant \( (\text{adjusted } p \text{ value } 0.0423, \text{adjusted } p \text{ value after Bonferroni correction } 0.0052) \), and was nonsignificant for colorectal cancer \( (\text{unadjusted } p \text{ value } 0.0423, \text{adjusted } p \text{ value } 0.5499) \) (Table I).

Odds ratios were further analysed by taking into account the age of disease onset \( (\leq 50, > 50) \). This split was arbitrarily chosen because early onset of malignancy suggests predominance of genetic over environmental factors in tumor development. There were no significant differences between early-onset lung cancers \( (5/70; 7.1\%) \) and late-onset cancers \( (29/427; 6.8\%) \). The A148T prevalence was slightly higher in late-onset colorectal cancers \( (5.1\%) \) than in early-onset cases \( (4.9\%) \) \( (\text{the number of patients with colon cancer diagnosed under age } 50 \text{ was less } (n = 122), \text{ thereby reducing the power of this result}) \).

We also compared subgroups of nonsmall cell lung cancers versus small cell lung cancers and left-side colorectal cancers versus right-side colorectal cancers (data not shown). We found no differences in A148T prevalence in these subgroups.

**Discussion**

Most of the patients in our case group were recruited from the Szczecin region, which is populated by ethnic Poles who immigrated to the region from throughout Poland after the Second World War, as ethnic German residents were relocated elsewhere. Our control group was drawn both from the adult population of Szczecin and from newborns in 10 cities throughout Poland. The frequency of the CDKN2A variant was similar in the newborn and

### Table 1 – Association Between A148T Variants and Selected Types of Cancer

<table>
<thead>
<tr>
<th></th>
<th>A148T</th>
<th>OR</th>
<th>95% CI</th>
<th>( p ) adjusted ( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total controls ( n = 3000 )</td>
<td>0 (0%) A/A</td>
<td>7 (3.1%) G/A</td>
<td>216 (96.9%) G/G</td>
<td>Allele A frequency 1.6%</td>
</tr>
<tr>
<td>Bladder ( n = 223 )</td>
<td>0 (0%) A/A</td>
<td>7 (3.1%) G/A</td>
<td>216 (96.9%) G/G</td>
<td>Allele A frequency 1.6%</td>
</tr>
<tr>
<td>Colon ( n = 724 )</td>
<td>0 (0%) A/A</td>
<td>37 (5.1%) G/A</td>
<td>687 (94.9%) G/G</td>
<td>Allele A frequency 2.6%</td>
</tr>
<tr>
<td>Stomach ( n = 246 )</td>
<td>0 (0%) A/A</td>
<td>8 (3.3%) G/A</td>
<td>238 (96.7%) G/G</td>
<td>Allele A frequency 1.6%</td>
</tr>
<tr>
<td>Larynx ( n = 396 )</td>
<td>0 (0%) A/A</td>
<td>17 (4.3%) G/A</td>
<td>379 (95.7%) G/G</td>
<td>Allele A frequency 2.1%</td>
</tr>
<tr>
<td>Ovary ( n = 340 )</td>
<td>0 (0%) A/A</td>
<td>12 (3.5%) G/A</td>
<td>328 (96.5%) G/G</td>
<td>Allele A frequency 1.8%</td>
</tr>
<tr>
<td>Lung ( n = 497 )</td>
<td>0 (0%) A/A</td>
<td>34 (6.8%) G/A</td>
<td>463 (93.2%) G/G</td>
<td>Allele A frequency 3.4%</td>
</tr>
<tr>
<td>Prostate ( n = 348 )</td>
<td>0 (0%) A/A</td>
<td>13 (3.7%) G/A</td>
<td>335 (96.3%) G/G</td>
<td>Allele A frequency 1.9%</td>
</tr>
<tr>
<td>Kidney ( n = 264 )</td>
<td>0 (0%) A/A</td>
<td>6 (2.3%) G/A</td>
<td>258 (97.7%) G/G</td>
<td>Allele A frequency 1.1%</td>
</tr>
<tr>
<td>Thyroid ( n = 173 )</td>
<td>0 (0%) A/A</td>
<td>3 (1.7%) G/A</td>
<td>170 (98.3%) G/G</td>
<td>Allele A frequency 0.9%</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma ( n = 162 )</td>
<td>0 (0%) A/A</td>
<td>6 (3.7%) G/A</td>
<td>156 (96.3%) G/G</td>
<td>Allele A frequency 1.9%</td>
</tr>
<tr>
<td>Pancreas ( n = 210 )</td>
<td>0 (0%) A/A</td>
<td>8 (3.8%) G/A</td>
<td>202 (96.2%) G/G</td>
<td>Allele A frequency 1.9%</td>
</tr>
</tbody>
</table>

The \( p \) value corresponds to the unadjusted \( p \) value of the Chi-square test. The adjusted \( p \) value after Bonferroni correction follows in parentheses (since we have previously examined melanoma and breast cancer cases, \( p \) values were multiplied by 13).
adult controls. Also, there was no statistical difference between the A148T change frequencies of the newborns recruited from the Szczecin metropolitan region and those of newborns from other Polish cities (data not shown). These findings and those published previously by our group suggest that the Polish population is homogeneous. However, to evaluate the problem of population stratification, it is necessary to determine the distribution of genotypes at a number of other unrelated loci in populations from throughout Poland. To evaluate the hypothesis that A148T could segregate with country of ancestry, other Slavic populations should be examined.

Multi-organ cancer predispositions are characteristic of other genes in DNA damage signaling pathways, including BRCA1, BRCA2, P53, NBS1 and CHEK2. Although any individual finding may be due to chance, overall our study suggests that the A148T variant of CDKN2A appears to be associated with an increased risk of developing cancer in several different organs. It appears to be associated with an increased risk of development of malignant melanoma and cancers of the breast and lung. Although statistically nonsignificant, we also observed a tendency of A148T overrepresentation among colorectal and laryngeal cancer cases. This is perhaps not surprising, since evidence from the literature indicates a possible association between CDKN2A and all of the aforementioned malignancies. The numbers of the cases were relatively small in this study and an association may have been missed as a result of limited study power. Especially interesting is the possibility of an association of A148T variant with modestly increased colorectal cancer risk. Separate chi-square evaluation revealed statistically significant difference (p = 0.0430), which became nonsignificant after the use of the Bonferroni correction.

To resolve this problem, it is necessary to perform a separate study based on examination of new subset of colorectal cancer cases.

We focused on the A148T variant, since other CDKN2A variants common in Polish population have been reported in our recent study to be not associated with melanoma risk. Since the A148T change has not been shown to alter protein function, yet appears to be associated with disease, it is most likely that this polymorphism is in linkage disequilibrium with another alteration that does affect protein function either by subtly altering its function or by reducing its expression. Indeed, there are several reports indicating that the A148T polymorphism is in linkage disequilibrium with a change in the promoter region at position P-493,19-20 and examination of this change in a subset of patients involved in this study revealed 100% concordance of the two changes. Regardless of it being pathogenic or not, we show herein that A148T could be used as a molecular marker of increased risk of development of such malignancies as melanoma, cancers of breast and lung in our population. Results of this study also point that it is necessary to repeat the data on new subsets of colon and laryngeal patients.

In conclusion, in this study, we have shown that the A148T allele is overrepresented in patients with melanoma and cancers, involving the breast and lung. This result suggests that the A148T allele is an important predictor of malignancy in the Polish population. We can not, at this time, say that the results are applicable to other populations. Large, well-controlled studies are now required to establish the full range of risks associated with CDKN2A founder alleles in different populations to estimate more precisely the corresponding disease likelihoods associated with diverse sites of malignancy.

References