Selenium and cancer prevention
Margaret Rayman
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The essential trace mineral, selenium, has been the focus of an increasing number of research studies since the 1996 report that it decreased the risk of some common cancers. Prospective studies have generally shown some benefit of higher selenium status on the risk of prostate, lung, colorectal, and bladder cancers but trials have had mixed findings, likely highlighting the fact that supplementation will only confer benefit if intake of a nutrient is inadequate. There is evidence for the involvement of a plethora of mechanisms in the anti-cancer effects of selenium including protection from oxidative damage to DNA and stimulation of DNA repair mechanisms, which may be particularly relevant to genetic cancers. Research effort has focussed on low molecular weight selenium species and selenoproteins as cancer preventive agents. The importance of selenoproteins has been demonstrated by the fact that single nucleotide polymorphisms in selenoprotein genes affect the risk of a number of cancers as does methylation of the promoter region of the GPx3 gene which inhibits expression of this protective selenoprotein. Selenium intake, and therefore status, varies tremendously across the world demonstrating both deficiency and toxicity. Trial evidence has suggested that supplementing those who already have adequate selenium intake and maximal selenoprotein activity/concentration with additional selenium may increase the risk of alopecia, dermatitis and the level of Fe and Se could be an important factor for lung cancer risk. We analyzed iron (Fe) level in serum of 77 lung cancer patients and 77 matched controls. We did not find difference in mean Fe level between cases and controls (1053.05 µg/l and 1059.39 µg/l). However, we found that Fe level in the lowest and highest quartiles was associated with a significant difference in mean Fe level between cases and controls (p < 0.0001). Compared to a serum selenium value in the lowest of four categories (< 80 µg/l) a selenium level in the highest category (> 80 µg/l) was associated with an odds ratio of 0.10 (95% CI 0.03 to 0.34; p = 0.0002) for lung cancer and 0.24 (95% CI 0.10 to 0.59; p = 0.002) for laryngeal cancer. In four selenoproteins studied here we found a modest associations of genetic variants in GPx1 and GPx4 with lung and TXNRD2 with laryngeal cancer risk. We analyzed iron (Fe) level in serum of 77 lung cancer patients and 77 matched controls. We did not find difference in mean Fe level between cases and controls (1053.05 µg/l and 1059.39 µg/l). However, we found that Fe level in the lowest and highest quartiles was associated with a significant lung risk enhancement when compared to a serum Fe level in the middle quartiles (OR 0.10 to 0.59; p = 0.002). Among laryngeal cancer cases, the mean selenium level was 64.8 µg/l, compared to a mean level of 76.3 µg/l for their matched controls (p < 0.0001). Compared to a serum selenium value in the lowest of four categories (< 80 µg/l) a selenium level in the highest category (> 80 µg/l) was associated with an odds ratio of 0.10 (95% CI 0.03 to 0.34; p = 0.0002) for lung cancer and 0.24 (95% CI 0.10 to 0.59; p = 0.002) for laryngeal cancer. In four selenoproteins studied here we found a modest associations of genetic variants in GPx1 and GPx4 with lung and TXNRD2 with laryngeal cancer risk.

Microelements as risk factors for cancer of the lung and larynx
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Selenium deficiency has been suggested by several studies to be associated with cancer risk. We conducted a case-control study in Szczecin, a region of northwestern Poland, on 86 cases of lung cancer, 87 cases of laryngeal cancer and an equal number of healthy controls. We studied the serum level of selenium and genotypes for four variants in four selenoprotein genes (GPX1, GPX4, TXNRD2 and SEP17) and the odds of being diagnosed with lung or laryngeal cancer. Among lung cancer cases, the mean selenium level was 63.2 µg/l, compared to a mean level of 74.7 µg/l for their matched controls (p < 0.0001). Among laryngeal cancer cases, the mean selenium level was 64.8 µg/l, compared to a mean level of 76.3 µg/l for their matched controls (p < 0.0001). Compared to a serum selenium value in the lowest of four categories (< 80 µg/l) a selenium level in the highest category (> 80 µg/l) was associated with an odds ratio of 0.10 (95% CI 0.03 to 0.34; p = 0.0002) for lung cancer and 0.24 (95% CI 0.10 to 0.59; p = 0.002) for laryngeal cancer. In four selenoproteins studied here we found a modest associations of genetic variants in GPx1 and GPx4 with lung and TXNRD2 with laryngeal cancer risk.

Selenium and gastrointestinal cancers risk
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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 4) A2-A3
Research suggests that selenium may influence the behavior of the cancer risk in two ways. As an antioxidant, selenium helps to protect the body against free radicals. Selenium may also prevent or slow tumor growth, as some breakdown products of selenium can inhibit tumor growth by enhancing immune cell activity and inhibition of tumor blood vessel development.

**Aim:** The aim of this study was to determine the level of selenium in blood serum as a potential marker of risk for cancers of the colon, stomach or pancreas.

**Material and methods:** The research material was a total of 110 samples of blood serum from people with cancer, diagnosed and confirmed in one of the organs: colon (67 cases), pancreas (30 cases) or stomach (13 cases) and 110 samples of blood serum derived from healthy individuals representing paired control group. The criteria adopted for pairing included: gender, year of birth (+/− 3 years), history of the occurrence of cancers in the family among first degree relatives and smoking status expressed in pack-years.

Selenium concentration in blood serum was determined using inductively coupled plasma mass spectrometry (ICP-MS) – Perkin Elmer. Validation: Seronorm™, Nycomed Pharma A/S, Oslo, Norway. The measurement accuracy was +/- 5% µg Se/l.

**Results:** Association between Se concentration and frequency of cancers in quartiles are presented in table 1. Statistical analyses are summarized in table 2.

**Conclusions:** 1. There is a very strong correlation between the level of selenium in serum and the risk of gastrointestinal cancers (pancreas, colon, stomach) in the Polish population. 2. Due to the risk of gastrointestinal cancers evaluated most people from the Polish population should increase the level of selenium in serum to approximately 80-100 µg/l.

3. Prospective studies can elucidate:
   a) the use of selenium measurements as markers of risk of above cancers, 
   b) possibility of lowering risk of the cancers of the colon, pancreas and stomach by supplementation of diet with selenium.

4. Assessment of the level of selenium may increase the effectiveness of many screening programs of gastrointestinal cancers, for example, the cost of detecting asymptomatic colorectal cancer by colonoscopy can be reduced dozen times.

### Table 1(abstract A3) Association between Se plasma concentration and risk of cancers analyzed.

<table>
<thead>
<tr>
<th>Cancer site</th>
<th>Quartile</th>
<th>Se concentration range (µg/l)</th>
<th>No. of Cases/ Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pancreas</strong></td>
<td>I</td>
<td>25,69-50,09</td>
<td>15/0</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>50,72-65,58</td>
<td>9/6</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>66,34-73,30</td>
<td>6/9</td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>74,07-113,89</td>
<td>0/15</td>
</tr>
<tr>
<td><strong>Colorectal and stomach</strong></td>
<td>I</td>
<td>15,92-56,75</td>
<td>35/5</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>57,3-67,91</td>
<td>22/18</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>68,13-78,07</td>
<td>12/28</td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>78,38-155,72</td>
<td>11/29</td>
</tr>
</tbody>
</table>

### Table 2(abstract A3) Results of statistical analyses of cancer site depending on Se concentration.

<table>
<thead>
<tr>
<th>Cancer site</th>
<th>Quartiles compared</th>
<th>Se concentration range (µg/l)</th>
<th>Cases/controls in compared groups</th>
<th>Fisher’s Exact Test</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pancreas</strong></td>
<td>I vs II</td>
<td>25,69-50,09 vs 50,72-65,58</td>
<td>15/0 vs 9/6</td>
<td>P       0.0017 21.2 1.07–421.11</td>
</tr>
<tr>
<td></td>
<td>I vs III</td>
<td>25,69-50,09 vs 66,34-73,30</td>
<td>15/0 vs 6/9</td>
<td>P       0.0007 45.3 2.2–899.53</td>
</tr>
<tr>
<td></td>
<td>I vs IV</td>
<td>25,69-50,09 vs 74,07-113,89</td>
<td>15/0 vs 0/15</td>
<td>&lt;0.0001 961 7.9–51,617</td>
</tr>
<tr>
<td><strong>Colorectal and stomach</strong></td>
<td>I vs III</td>
<td>15,92-56,75 vs 57,3-67,91</td>
<td>(5)*35/5 vs (4)*22/18</td>
<td>0.0026 5.7 1.86–17,66</td>
</tr>
<tr>
<td></td>
<td>I vs III</td>
<td>15,92-56,75 vs 68,13-78,07</td>
<td>(5)*35/5 vs (1)*12/28</td>
<td>&lt;0.0001 16,33 5.14–51,89</td>
</tr>
<tr>
<td></td>
<td>I vs IV</td>
<td>15,92-56,75 vs 78,38-155,72</td>
<td>(5)*35/5 vs (3)*11/29</td>
<td>&lt;0.0001 18,46 5.76–59,25</td>
</tr>
</tbody>
</table>

*stomach cancer cases
Table 1(abstract A4) Correlation between breast cancer risk and serum selenium concentration in carriers of GPX1 nCC genotype:

<table>
<thead>
<tr>
<th>BRCA1 gene mutation carriers</th>
<th>Quartiles</th>
<th>Concentration Se (µg/l)</th>
<th>No. cases</th>
<th>No. controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast (BRCA1)</td>
<td>I</td>
<td>52.37 – 70.45</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>72.45 – 78.03</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>78.68 – 85.5</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>88.5 – 741.33</td>
<td>3</td>
<td>5</td>
</tr>
</tbody>
</table>

Unselected breast cancer

<table>
<thead>
<tr>
<th>Cancer</th>
<th>Quartiles</th>
<th>Concentration Se (µg/l)</th>
<th>No. cases</th>
<th>No. controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unselected breast cancer</td>
<td>I</td>
<td>49.73 – 72.51</td>
<td>29</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>72.63 – 82.73</td>
<td>20</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>83.00 – 95.76</td>
<td>27</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>96.74 – 160.97</td>
<td>26</td>
<td>30</td>
</tr>
</tbody>
</table>

The nCC genotype (Table 1) is around 75µg/l of serum and for carriers of GPX1 CC the optimal selenium level is 120µg/l.

Conclusions: Selenium, depending on Se concentration, can cause cancer or prevent against cancer.

- The effect of selenium depends on selenoprotein genotypes and concentration of selenium in the body/diet.
- Generally the optimal concentration of selenium for women is in range 100 – 120µg/l of serum.
- For smokers (BRCA1 mutation carriers and unselected breast cancer) with genotype nCC in GPX1 gene the optimal concentration of selenium is around 75µg/l of serum.
- The results of association studies require confirmation by a prospective observation of large groups of patients.
- Investigation on larger number of patients is needed especially valuable may be observations of women with increased risk of cancers.

A5
Iron and breast cancer risk
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Read-Gene SA and Pomeranian Medical University, Szczecin, Poland
Hereditary Cancer in Clinical Practice 2012, 10(Suppl 4):A5

The aim of the study is identification of correlations between the serum concentrations of iron and the risk of breast cancer among female BRCA1 mutation carriers and unselected breast cancer patients.

The first group (99 triplet) selected for the trial were Polish women, positive for at least one of three founder mutations in BRCA1 gene dominating in Poland (5382insC, C61G, 4153delA). Serum was collected at the time of breast cancer diagnosis. Persons with detected tumor were considered as cases and the others were considered as controls. One case and two controls were paired regarding many criteria (e.g. age, family cancer history, cigarettes smoking, adnexectomy status) to achieve the maximum of similarity between them.

The subjects of the second group (28 triplet) selected for the trial were Polish women, positive for at least one of three founder mutations in BRCA1 gene. Serum was collected 1-2 years before breast cancer diagnosis. One case and two controls were matched for year of birth, past history of cancer, adnexectomy status and cigarettes smoking to achieve the maximum of similarity between them.

The subjects of the third group were unselected cancer for the trial were Polish women. Serum was collected during breast cancer diagnosis before treatment. One case and one control were matched for year of birth (+/- 3 years), number and location of cancer among 1º relatives, smoking-the number of pack years (+/- 10%), adnexectomy status, CHEK2 mutation.

The proportion of cases and control in the first quartile was taken as a reference to calculate the odds ratio, confidence interval and p-value of the multivariate conditional logistic regression. The iron was quantitatively measured by ICP-MS (Inductively Coupled Plasma Mass Spectrometry), (model Elan DRC-e 6100 th, PerkinElmer). This study shows that concentrations levels of iron in blood serum are a strong factors associated with an additionally increased risk of breast cancer among BRCA1 mutation carriers and unselected breast cancer patients.

For iron concentration in BRCA1 carriers (serum collected at the time of breast cancer diagnosis) all quartiles above the first one had a decreased risk of breast cancer. The results are shown in Table 1. For iron concentration in BRCA1 carriers (serum collected 1-2 years before breast cancer diagnosis) the last quartile had a decreased risk of breast cancer.

Table 1 (abstract A5) Association between Fe serum concentration and risk of breast cancer in BRCA1 carriers (serum collected at the time of breast cancer diagnosis).

<table>
<thead>
<tr>
<th>Fe (µg/l)</th>
<th>Cases (n=99)</th>
<th>Controls (n=198)</th>
<th>OR</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>370,95 – 977,64</td>
<td>34 (34,3%)</td>
<td>40 (20,2%)</td>
<td>1,00</td>
<td>-</td>
</tr>
<tr>
<td>977,64 – 1262,78</td>
<td>24 (24,2%)</td>
<td>50 (25,3%)</td>
<td>0.565</td>
<td>0.01229</td>
</tr>
<tr>
<td>1262,78 – 1571,11</td>
<td>24 (24,2%)</td>
<td>50 (25,3%)</td>
<td>0.565</td>
<td>0.13885</td>
</tr>
<tr>
<td>1571,11 - 4756,15</td>
<td>17 (17,1%)</td>
<td>58 (29,3%)</td>
<td>0.345</td>
<td>0.00823</td>
</tr>
</tbody>
</table>

Table 2 (abstract A5) Association between Fe serum concentration and risk of breast cancer in BRCA1 carriers (serum collected 1-2 years before breast cancer diagnosis).

<table>
<thead>
<tr>
<th>Fe (µg/l)</th>
<th>Cases (n=27)</th>
<th>Controls (n=56)</th>
</tr>
</thead>
<tbody>
<tr>
<td>313,90 – 945,79</td>
<td>9 (33,3%)</td>
<td>12 (21,4%)</td>
</tr>
<tr>
<td>954,79 – 1157,15</td>
<td>9 (33,3%)</td>
<td>12 (21,4%)</td>
</tr>
<tr>
<td>1158,32 – 1437,54</td>
<td>5 (18,5%)</td>
<td>16 (28,6%)</td>
</tr>
<tr>
<td>1460,00 – 2193,10</td>
<td>4 (14,8%)</td>
<td>16 (28,6%)</td>
</tr>
</tbody>
</table>
The strongest result and lowest cancer risk was for Fe > 1350 µg/l (OR = 0.248, p = 0.019).

For iron concentration in unselected breast cancer patients (serum was collected during breast cancer diagnosis before treatment) all quartiles were statistically insignificant. The results are shown in Table 3.

Individuals classified in the III quartile had lower risk of breast cancer however results were statistically insignificant.

The strongest result and lowest cancer risk was for Fe > 1000 µg/l (OR = 0.6698, p = 0.25554).

For iron concentration in unselected breast cancer patients (serum was collected during breast cancer diagnosis before treatment) before 55 years old, third quartile had a decreased risk of breast cancer. The results are shown in Table 4.

Individuals classified in the III quartile had lower risk of breast cancer (OR = 0.248, p = 0.019);

The strongest result and lowest cancer risk was for Fe concentration higher than 987 µg/l (III quartile); max 1285 µg/l (III quartile).

Table 3 (abstract A5) Association between Fe serum concentration and risk of breast cancer

<table>
<thead>
<tr>
<th>Fe (µg/l)</th>
<th>Cases (n=151)</th>
<th>Controls (n=151)</th>
</tr>
</thead>
<tbody>
<tr>
<td>86.47 – 813.58</td>
<td>43 (28.47%)</td>
<td>32 (21.19%)</td>
</tr>
<tr>
<td>813.92 – 992.11</td>
<td>40 (26.49%)</td>
<td>35 (23.18%)</td>
</tr>
<tr>
<td>993.06 – 1265.75</td>
<td>35 (23.18%)</td>
<td>41 (27.15%)</td>
</tr>
<tr>
<td>1268.27 – 3676.38</td>
<td>33 (21.85%)</td>
<td>43 (28.48%)</td>
</tr>
</tbody>
</table>

Table 4 (abstract A5) Association between Fe serum concentration and risk of cancer in group of patients <55 years old

<table>
<thead>
<tr>
<th>Fe (µg/l)</th>
<th>Cases (n=59)</th>
<th>Controls (n=62)</th>
</tr>
</thead>
<tbody>
<tr>
<td>127.58 – 725.31</td>
<td>19 (32.2%)</td>
<td>11 (17.7%)</td>
</tr>
<tr>
<td>752.60 – 987.19</td>
<td>17 (28.8%)</td>
<td>13 (21.0%)</td>
</tr>
<tr>
<td>987.19 – 1285.18</td>
<td>9 (15.3%)</td>
<td>21 (33.9%)</td>
</tr>
<tr>
<td>1294.72 – 2584.76</td>
<td>14 (23.7%)</td>
<td>17 (27.4%)</td>
</tr>
</tbody>
</table>

The aim of study was to analyze possible association between serum zinc level and breast cancer risk in BRCA1 mutation carriers and noncarriers.

Materials and methods: The studies have been performed on 3 independent groups: (1) 119 unselected breast cancer patients matched 1:1 with 119 unaffected controls (carriers of BRCA1 mutation have been excluded), (2) 99 breast cancer cases (serum collected at the moment of diagnosis, before treatment) and 198 matched 1:2 unaffected controls, (3) 27 breast cancer cases (serum collected 1-2 years before diagnosis) and 53 controls matched 1:2. All individuals in groups (2) and (3) were carriers of BRCA1 mutation.

Zinc is a micronutrient, which is essential for human health, playing role as a cofactor of enzymes such as dehydrogenases, peptidases and component of zinc finger domains. In organism zinc is involved in metabolic pathways, immune processes, maintaining ion balance between cellular components and genetic disorders, including heart disease, stroke and cancers.

Table A6

| Katarzyna Durda¹, Anna Jakubowska², Grzegorz Sukiennicki, Magdalena Muzynska, Katarzyna Jaworska-Bieniek¹, Katarzyna Kaczmarek, Tomasz Huzarski, Pablo Serrano-Fernandez, Tomasz Byrski, Jacek Gronwald, Magdalena Muszyńska³, Anna Jakubowska¹ |

A7 Folic acid and breast cancer risk

Katarzyna Durda¹, Katarzyna Jaworska-Bieniek³, Krzysztof Kąklewski¹, Jan Lubierski¹, Anna Jakubowska¹

¹International Hereditary Cancer Centre, Department of Genetics and Pathology, Pomeranian Medical University, Szczecin, Poland; ²Postgraduate School of Molecular Medicine, Warsaw Medical University, Warsaw, Poland; ³Hereditary Cancer in Clinical Practice 2012, 10(Suppl 4): A7

Recent studies indicate that the selected micro- and macro-elements and vitamins may significantly influence the risk of cancer. Folic acid is a vitamin B which plays an important role in several processes in organism. Folate are important cofactors in the transfer and utilization of one-carbon-groups and play a key role in the remethylation of methionine thus providing essential methyl groups for numerous biological reactions. Furthermore, folates donate one-carbon units in the process of DNA-biosynthesis with implications for the regulation of gene expression, transcription, chromatin structure, genomic repair and genomic stability. Deficiency of folic acid has been reported to be associated with numerous disorders, including heart disease, stroke and cancers.

The MTHFR gene produces a key enzyme in folate metabolism which catalyses the reactions essential for nucleotide biosynthesis and DNA methylation.

The aim of study was to analyze an association of folic acid concentrations and genetic variants in the MTHFR gene with breast cancer risk in patients with BRCA1 mutation.

Study group consisted of 155 breast cancer patients and 155 healthy women from the paired control group matched to cases by year of birth, cancer family history, axillaryectomy, smoking. From all individuals blood sample was collected and from cancer cases was taken before treatment. Folic acid concentration was quantitatively measured in blood plasma by HPLC chromatography (Flexar HPLC, Perkin Elmer). Two functional SNPs in the MTHFR gene, 677 C>T (rs1801133) and 1298 A>C (rs1801131), both associated with reduced enzyme activity, have been tested by TaqMan on LightCycler 480 (Roche Diagnostic). Individuals were divided into four quartiles depending on folic acid concentration and number of cases and controls in each quartile was compared. Analysis was made depending on menopausal status defined as ≤ 50 and >50 years old.

In a group of >50 years old, individuals classified in the first quartile (<17.35 µmol/l) had a lower risk of breast cancer than patients with higher folate level. Whereas in a group of ≤50 years old, individuals classified in the 3 quartile (24.35 – 31.88 µmol/l) had a significantly lower risk of breast cancer than those with folate level between 32.02 – 54.42 µmol/l. Analysis of correlation between the level of folic acid and genetic variants 677 C/T and 1298 A/C in the MTHFR gene performed in 2 groups (>50 and ≤50 years) revealed:

- for carriers of 1298 nCC and ≤50 years old significantly lower risk of breast cancer in individuals classified between 16.6-31.88 µmol/l in comparison to patients with lower and higher folate level.
The germline mutations in BRCA1/2 genes are the most significant and well characterized genetic risk factors for breast and/or ovarian cancer. Detection of mutations in these genes is an effective method of cancer prevention and early detection. Different ethnic and geographical regions may have different BRCA1 and BRCA2 mutation spectrum and prevalence due to founder effect. The population of Lithuania has over several centuries undergone limited mixing with surrounding populations and is mostly of indigenous Baltic origin, which is different from Slavs. The aim of our study was to assess BRCA1/2 mutational profile in Lithuanian population.

**Methods:** We performed comprehensive mutation analysis of BRCA1/2 genes in 605 unrelated breast and/or ovarian cancer patients (with/without family history) and predictive unaffected patients (with family history) using high resolution melting (HRM) screening (Light Cycler 480/480 Light Scanner 384) followed by direct sequencing (ABI 3500) and MLPA for large genomic rearrangements (LGRs).

**Results:** Overall, we have identified 25 different mutations (16 in BRCA1 and 9 in BRCA2 genes). Seven frequent mutations: BRCA1 gene: (c.4035delA, c.4035delA, c.4789C>G, del 1-3ex) comprised 48%, 29%, 7.7%, 3.5%, 2.1%, 1.4% and 1.4% respectively of all BRCA1 mutations; a single BRCA2 mutation (c.658delG) comprised 43% of all mutations in this gene. Five novel BRCA1 (c.4516delG, c.4516delG, c.550delC, c.16-19ex, del 3-12ex) and 4 novel BRCA2 genes mutations (c.640G, 641A, 641G, 641delA) were identified; 3 different LGRs (del 1-3ex, del 16-19ex, del 3-12ex) were found in BRCA1. The most common c.4035delA (48% of all BRCA1/2 mutations) appears to be true Lithuanian (Baltic) founder mutation and haplotype data confirmed ancient origin of this mutation c.a. 62 generations ago.

**Conclusions:** Characterization of BRCA1/2 mutational profile in Lithuania enabled us to develop screening protocol using HRM for 8 common BRCA1/2 point mutations, which comprise 88% of all mutations detected in our country. This knowledge will provide more efficient approach for the individualization of genetic testing affordable for all breast/ovarian patients and their relatives.

#### A8
**Arsenic (As) and breast cancer risk**

Magdalena Muszyńska, Katarzyna Jaworska-Bieniek, Katarzyna Durda, Grzegorz Sukienicki, Tomasz Gromowski, Anna Jakubowska, Antoni Morawski, Jan Lubriński

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**Hereditary Cancer in Clinical Practice 2012, 10(Suppl 4):A8**

All subjects analyzed in the study were divided into two groups. The subjects from the first group were Polish women, positive for at least one of three founder mutations in BRCA1 gene dominating in Poland (538insC, C61G, 4153delA). In the second group were Polish women, breast cancers, unselected but negative for BRCA1 gene mutation. Persons with detected tumor were considered as cases and the others were considered as controls. In the case first group one case and two controls, and in the case second group one case and one control, were paired regarding many criteria (e.g. age, family cancer history, cigarettes smoking), adnexectomy to achieve the maximum of similarity between them. Arsenic was quantitatively measured in diluted serum samples by inductively coupled plasma mass spectrometry (ICP-MS) using mass spectrometer (Elan DRC-e, PerkinElmer) in DRC mode with methane as a reaction gas, for removing polyatomic interferences in measurement.

Arsenic gave statistically significant differences in the disease risk when comparing the proportion of controls and cases in a certain quartile with the same proportion in the first quartile. Individuals classified in the second (3.6 µg/l – 4.3 µg/l), third (4.3 µg/l – 5.7 µg/l) and fourth quartile (5.7 µg/l – 50 µg/l) had a significantly higher risk of breast cancer (OR=1.7, p=0.016, OR=2.25, p=0.007; OR=1.4, p=0.04, respectively) than those in the first quartile (1.1 µg/l – 3.6 µg/l) in BRCA1 gene mutation carriers. In the second group surprisingly lower risk of breast cancer was observed among individuals classified in the second quartile (2.01 µg/l – 2.97 µg/l) in comparison with fourth quartile (4.11 µg/l – 9.14 µg/l) (p=0.054, OR=2.4). Additionally, ratio between arsenic and selenium was analyzed. For the first group the ratios with statistically significant differences observed in quartiles in the disease risk are shown in Table 1. In the second group there were no statistically significant differences observed.

<table>
<thead>
<tr>
<th>Table 1 (abstract A8) Ratios between analyzed elements and selenium among BRCA1 mutation carriers</th>
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<tr>
<td><strong>As/Se</strong></td>
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<td>0.016 - 0.044</td>
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#### A9
**BRCA1 testing in Lithuania**

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**Hereditary Cancer in Clinical Practice 2012, 10(Suppl 4):A9**

**Background:** The germline mutations in BRCA1/2 genes are the most significant and well characterized genetic risk factors for breast and/or ovarian cancer. Detection of mutations in these genes is an effective method of cancer prevention and early detection. Different ethnic and geographical regions may have different BRCA1 and BRCA2 mutation spectrum and prevalence due to founder effect. The population of Lithuania has over several centuries undergone limited mixing with surrounding populations and is mostly of indigenous Baltic origin, which is different from Slavs. The aim of our study was to assess BRCA1/2 mutational profile in Lithuanian population.

**Methods:** We performed comprehensive mutation analysis of BRCA1/2 genes in 605 unrelated breast and/or ovarian cancer patients (with/without family history) and predictive unaffected patients (with family history) using high resolution melting (HRM) screening (Light Cycler 480/480 Light Scanner 384) followed by direct sequencing (ABI 3500) and MLPA for large genomic rearrangements (LGRs).

**Results:** Overall, we have identified 25 different mutations (16 in BRCA1 and 9 in BRCA2 genes). Seven frequent mutations: BRCA1 gene: (c.4035delA, c.4035delA, c.4789C>G, del 1-3ex) comprised 48%, 29%, 7.7%, 3.5%, 2.1%, 1.4% and 1.4% respectively of all BRCA1 mutations; a single BRCA2 mutation (c.658delG) comprised 43% of all mutations in this gene. Five novel BRCA1 (c.4516delG, c.4516delG, c.550delC, c.16-19ex, del 3-12ex) and 4 novel BRCA2 genes (c.640G, 641A, 641G, 641delA) were identified; 3 different LGRs (del 1-3ex, del 16-19ex, del 3-12ex) were found in BRCA1. The most common c.4035delA (48% of all BRCA1/2 mutations) appears to be true Lithuanian (Baltic) founder mutation and haplotype data confirmed ancient origin of this mutation c.a. 62 generations ago.

**Conclusions:** Characterization of BRCA1/2 mutational profile in Lithuania enabled us to develop screening protocol using HRM for 8 common BRCA1/2 point mutations, which comprise 88% of all mutations detected in our country. This knowledge will provide more efficient approach for the individualization of genetic testing affordable for all breast/ovarian patients and their relatives.
A11

A method of rapid and cost-effective screening for small mutations in patients with Peutz-Jeghers Syndrome

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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 4):A11

Peutz-Jeghers syndrome (PJS, MIM # 175200) is a rare, hereditary predisposition characterized by the occurrence of hamartomatous polyps in the gastrointestinal tract, mucocutaneous pigmentation, and increased risk of cancer in multiple internal organs. The incidence of the syndrome, depending on the studied population, has estimated range from 1:2,500 even up to 1:300,000 births. PJS is an autosomal dominant disease and in most of the cases is caused by mutations in the STK11 gene (MIM # 602216) located on a small arm of chromosome 19 at position 19p3.3.

The majority of causative DNA changes identified in patients with PJS are small mutations, therefore, developing a rapid and cost-effective method of their detection is a key aspect in the advancement of genetic diagnostics of patients suffering from PJS. In our study we developed a methodology of detection of small mutations in entire coding sequence of the STK11 gene based on High Resolution Melting analysis (HRM). In our group of 41 families with PJS small mutations of the STK11 gene were detected in 22 families (54%). In the remaining cases, where large rearrangements were not detected using MLPA, all of the coding exons were sequenced. However, this did not allow detection of any additional mutations. Therefore, the developed methodology of searching for small mutations using HRM allowed to detect all the STK11 gene sequence changes occurring in our group of patients. The study was financed by the Ministry of Education and Science, Poland, grant number N402481537.

A12

Male breast cancer in the prophylactics programme by the Ministry of Health of Poland

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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 4):A12

The prophylaxis programme for the families with high hereditary risk of malignant cancers organized and guided by the Ministry of Health of Poland. Our data are based on the activity of the ZOZ “SALVE”, one of three units operating in the Lodz district in Poland. Over 600 questionnaire producers by patients with problems of in-family cases of cancer (including ovary cancers and/or breast cancer) were collected between mid-2010 to the end of the 2011. Four cases of male breast cancer were recorded and screened across the clinically recorded family data for these patients. It appeared that these cases could not be fully explained in accordance with the current concept of the family cases of cancers. These discrepancies could be either related to the faulty selection criteria or to the highly differentiated male population suffering the breast cancer. The diagnostic potential within the “Module 1: early detection of malignant cancers in hereditary high-risk families with ovary cancer and breast cancer” is discussed and evaluated in practical terms including regulatory/legislation aspects. Supported by the Ministry of Health of Poland.

A13

Constitutional methylatation of cancer-related and selenoprotein coding genes in breast carcinoma in Polish population

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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 4):A13

Recently, much attention is paid to the phenomenon of gene’s hypermethylation in the peripheral blood (PB) and its involvement in the pathology of cancer. Breast cancer is a complex disease driven by multiple factors including both genetic and epigenetic processes. Genetic changes associated with breast cancer are not completely known, but epigenetic mechanisms involved in this disease seem to play an important role in its pathophysiology. An aberrant methylation in the promoter regions of genes involved in cancer induction and promotion, like BRCA1, BRCA2, ATM, MLH1 and ESRI, may be of particular importance in breast cancer.

A very interesting class of genes, involved in selenium metabolism is under investigation with respect to different kind of cancers including breast cancer. Proteins coded by these genes, e.g. glutathione peroxidases, thioredoxin reductases or other selenoproteins are involved in variety of biological processes, ranging from DNA synthesis to protection against oxidative stress and may be related to breast cancer risk.

The aim of our study was to analyze methylation of genes involved in selenium metabolism (GPX1, GPX4, TXNBD1, SEP15 and SELT) and other cancer related genes (BRCA1, BRCA2, ATM, MLH1 and ESRI) in DNA isolated from PB of breast cancer patients or unaffected individuals.

A study was conducted on 30 female BRCA1 mutation carriers, 30 female CHEK2 mutation carriers and 36 unselected breast cancer patients negative for recurrent Polish BRCA1/BRCA2 mutations and with specific pathological characteristics: medullary or atypical medullary breast cancer or ER, PR and HER negative tumors. Methylation of BRCA1 gene was additionally analyzed in group of 36 healthy controls, age-inherited to unselected breast cancer patients. Methylation analysis was done by MS-HRM technique.

We observed promoter methylation of BRCA1 (20 samples), ESRI (7 samples) and MLH1 (3 samples) genes. BRCA1 gene methylation was detected in 1 patient with CHEK2 and 4 patients with BRCA1 mutation. We found strong statistically significant difference of BRCA1 gene methylation in unselected breast cancer patients (15 patients) and unaffected age-matched controls (2 individuals): OR=12.1, p-value=0.0009. Selenoprotein coding genes GPX1 and GPX4 shows complete methylation while TXNBD1 and SELT show methylation in ~10% of the studied group.

Conclusion: Constitutional methylation (in peripheral blood) of BRCA1 gene seems to be a strong factor risk of breast cancer with specific pathological characteristic.

A14

GWAS markers in diagnostics of breast cancer risk

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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 4):A14

Breast cancer belongs to the most common malignancy diagnosed in women. The major inherited susceptibilities to breast cancer are germline mutations in high risk genes, e.g. BRCA1, BRCA2, TP53, PALB2, RAD51C, RAD51D (5-10%), mutations in moderate risk genes, e.g. CHEK2, ATM, NBS1 (10-20%) which however, explain only a small number of breast cancer cases. The latter are alterations in low risk genes and other possible changes in genome, e.g. constitutional methylation of BRCA1.

Genome – Wide Association Study (GWAS) was thought to help in identification of low risk alleles associated with the risk of particular disease. Up to know 14 GWAS conducted on Caucasian and 6 GWAS on Asian population (http://www.genome.gov/gwastudies/index.cfm?pageld=26525384#searchForm) have been performed on breast cancer cases and 21 variants associated with modest risk of BC (OR <1.3) have been identified.

In 2005 have been initiated Breast Cancer Association Consortium (BCAC), an international multidisciplinary forum of investigators interested in the inherited risk of breast cancer. The aim of the consortium is to combine data from many studies in order to identify genes/SNPs and provide a reliable assessment of the breast cancer risks. Currently the BCAC gathers 69 centers from 29 countries, including Poland, and has access to demographic, clinical and epidemiological data from over 76,000 breast cancer patients and 83,000 unaffected women. In GWAS performed by BCAC 14 SNPs have been identified to be associated with modest risks of breast cancer(per-allele ORs<1.3).

The aim of this study was to assess usefulness of SNPs identified in BCAC GWAS in diagnostics of breast cancer risk in Polish population. We found that 6 out of 14 SNPs identified in BCAC GWAS were associated with breast cancer risk in Polish patients. Four additional SNPs have been found to be associated with moderate risk of breast cancer in Polish study.
Overall, out of 10 SNPs strongest in Polish study 3 seem to be valuable markers for breast cancer diagnostics in Poland: OR 1.91, 95%CI 1.39-2.65, p = 0.0001 for carriers of 2 risk alleles and OR 3.2, 95%CI 1.87-5.47, p < 0.0001 for carriers of 3 risk allele.

A15

BRCA1 mutation in the Triple- Negative Breast Cancer Group
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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 4):A15

Introduction: Approximately 75% of BRCA1 mutation-related breast cancers are triple- negative breast cancers (TNBC). Recent studies have found the prevalence of BRCA1 founder mutations in patients with TNBC and suggests BRCA1 mutation testing for all patients with TNBC regardless of family history and age at diagnosis of breast cancer.

Aim of the study: To investigate the prevalence of germline BRCA1 founder mutations in patients with TNBC and to find out appropriate indications for BRCA1 mutation testing in patients with TNBC.

Material and methods: 332 unslected patients with invasive breast cancer diagnosed between 2006-2011 were identified from Pauls Stradins Clinical University Hospital, Riga East University hospital databases and clinical data from medical records were retrospectively analyzed. All patients were tested for the two common founder mutations in BRCA1 (1533delA and 5382insC) in Latvia using a multiplex- specific polymerase chain reaction (PCR) assay.

Results: Of the 332 patients, 100 (30.1%) were identified as having TNBC, the remaining patients 232 (69.9%) were identified as having other breast cancer subtypes. Among patients with TNBC the BRCA1 mutations prevalence was 17% (17 of 100 cases) compared to other patients' group with 0.86% (2 of 232 cases) BRCA1 mutation prevalence (P = 0.001). In the TNBC group BRCA1 mutation carriers were significantly younger at diagnosis than non-carriers (median age, 53.6 years versus 57 years, respectively; P = 0.043). The vast majority of BRCA1 mutations within the TNBC group were diagnosed in women under 50 years of age- in 10 cases (58.8%), in 5 cases (29.4%) BRCA1 mutations were detected between 50 and 65 years of age and in 2 cases (11.8%) - in patients older than age 65 years. In 113 of 332 (34%) patients without family history of cancer 2 of 19 BRCA1 mutations were found, in 219 (66%) of 332 patients in 5 cases (29.4%) BRCA1 mutations were detected.

Conclusion: Our study data indicates a high prevalence of BRCA1 mutation in TNBC and suggests BRCA1 mutation testing for all patients with TNBC regardless of family history and age at diagnosis of breast cancer.

A16

Systemic therapy for hereditary cancer
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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 4):A16

The history of specific therapy for hereditary tumors dates back to mid 1980s and involves a number of reports demonstrating regression of familial colon polyps upon administration of sulindac. Virtually no clinical studies on other hereditary cancer types were available until the year 2009, when Bryski et al. presented the data on unprecedented sensitivity of BRCA1-associated breast malignancies to cisplatin. This breakthrough has revived interest to the treatment of cancer in germ-line mutation carriers. Recent trials and clinical observations have confirmed the efficacy of platinum agents and PARP inhibitors in BRCA1/2-driven breast, ovarian and pancreatic carcinomas. Pegylated liposomal doxorubicin may be considered as a promising treatment option for BRCA1/2-related ovarian cancer after the failure of platinum-containing therapy. Several novel drugs have been recently introduced in the management of rare familial tumor syndromes. Vandetanib, a low-molecular weight RET kinase inhibitor, demonstrated substantial efficacy in the treatment of hereditary and sporadic medullary thyroid cancer. Vismodegib, an inhibitor of SMO oncoprotein, caused regression of basal-cell carcinomas in patients with Gorlin syndrome. Down-regulation of mTOR kinase by everolimus has been successfully used for the therapy of subependymal giant-cell astrocytomas in patients with tuberous sclerosis. The achievements in the prevention, diagnostics and treatment of hereditary cancers may serve as an excellent example of translational medicine.

A17

Cisplatin in breast cancer treatment in BRCA1 carriers
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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 4):A17

Experimental data suggest that BRCA1 related breast cancer may have increased sensitivity to platinum-based chemotherapy, but clinical data are limited. Herein we summarize our clinical observations on treatment with cisplatinum of BRCA1 mutation carriers affected with breast cancer.

A) Neoadjuvant therapy: Eighty women with breast cancer and a BRCA1 mutation with stage I, II, and III breast cancer between December 2006 and February 2012 were entered into this study. Patients were treated with cisplatin 75 mg/m2 intravenously every three weeks for four cycles. After chemotherapy, patients underwent surgery and were assessed for pathologic response in both the breast and axillary lymph nodes. Pathologic complete response was observed in 63% of women. Conclusions: Platinum-based chemotherapy is effective in a high proportion of patients with BRCA1-associated breast cancers. Clinical trials are warranted to determine the optimum treatment for this subgroup of breast cancer patients.

B) Treatment of metastatic breast cancer: Between July 2007 and January 2009, in a phase II, open-label study, 20 patients with metastatic breast cancer who carried a mutation in BRCA1 were treated with cisplatin 75 mg/m2 intravenously every 3 weeks as part of a 21-day cycle for 6 cycles. Restaging studies to assess response were performed after cycles 2 and 6, and every three months thereafter. Overall response rate was 80%; nine patients experienced a complete clinical response (45%) and seven experienced a partial response (35%). Overall survival was 80% at one year, 60% at two years and 25% at three years. Four of the 20 patients are alive four years after initiating treatment. The median time to progression was 12 months. The median survival from the start of cisplatinum treatment was 30 months. Cisplatin-related adverse events, including nausea (50%), anemia (5%) and neutropenia (35%) were mostly mild to moderate in severity.

Conclusions: This phase II study demonstrates that cisplatin chemotherapy has high activity in women with a BRCA1 mutation and metastatic breast cancer and is generally well tolerated.

A18

Screening with Magnetic Resonance Imaging in women at low and intermediate risk of breast Cancer
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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 4):A18
Purpose: The addition of MRI to mammography and ultrasound for breast cancer screening has been shown to improve screening sensitivity in high risk women (i.e., those with a BRCA mutation). Here we evaluate the addition of MRI to conventional screening (ultrasound and mammography) for women at average or intermediate risk of cancer.

Patients and Methods: From 2008 to 2011, 2995 women, aged 40 to 65 years with no previous history of breast cancer were enrolled in a prospective screening trial consisting of two annual rounds of MRI, ultrasound, and mammography. 356 women had a CHEK2 mutation, 458 women had a first-degree relative with breast cancer and 2269 women had neither risk factor. Subjects were followed for incident cancer for one year from the date of the second screening examination.

Results: In this cohort of 2995 women, 21 invasive epithelial cancers, one angiosarcoma and four cases of DCIS were identified over a two-year period. Of the invasive cancers, 20 were screen-detected and one was an interval cancer. Of the 21 invasive cancers detected in the cohort, 14 (67%) were less than 2 cm and 16 (76%) were node-negative. The sensitivity of MRI was 91.7%, which was far greater than the number incurred by either mammography (n = 35) or by ultrasound (n = 57). No cancer was identified by mammography that was not also identified by MRI, but one cancer was detected by ultrasound that was missed by MRI. Of the 19 cancers that were detected by MRI, 17 were also detected by ultrasound or mammography and two were detected by MRI alone.

Conclusion: In terms of sensitivity, MRI is superior or similar to the combination of mammography and ultrasound for screening of women at low or intermediate risk of breast cancer. However, because of the additional costs incurred and the number of biopsies required in order to detect a few additional breast cancers, MRI screening is probably not warranted outside of high-risk populations.

Acknowledgments: Wałęsa K., Chodyźrka I., Putresza E.

A20
The prevalence of CHEK2 and CYP1B1 mutations/polymorphisms in urinary bladder cancer
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Purpose: To estimate and to compare ten-year survival rates in unselected patients with early-onset breast cancer, with and without a BRCA1 mutation. To identify prognostic factors among unselected BRCA1-positive breast cancer patients.

Patients and methods: 3354 women who were diagnosed with stage I to stage III breast cancer were enrolled, of which at least 50 years of age, between January 1996 and December 2006 were contributed from 17 clinical centers in Poland. All patients were offered genetic testing for three founder mutations in BRCA1 (5382insC, C61G, 4184delA). Information on tumour characteristics at presentation and on treatments received was retrieved by reviewing the medical records. Mortality and dates of death were obtained by linkage to the vital statistics database of the Polish Ministry of Administration and Internal Affairs. Survival curves for the mutation-positive and mutation-negative sub-cohorts were constructed using Kaplan–Meier statistics and compared. Predictors of survival were determined using the Cox proportional hazards method.

Results: 3354 patients were enrolled in the study, of whom 234 (7.0%) were found to carry a BRCA1 founder mutation. The average age of diagnosis was 44 years (range 21 to 50 years). The ten-year survival for mutation carriers was 80.9% (95% CI 75.4% to 86.4%) and for non-carriers was 82.1% (95% CI 80.5% to 83.7%). After adjusting for other prognostic variables, the hazard ratio associated with carrying a BRCA1 mutation was 1.40 (95% CI: 0.99 to 1.99). Among BRCA1 mutation carriers, in the multivariable analysis, positive lymph nodes were a strong predictor of mortality (HR = 4.6; 95% CI: 1.00 to 20.20). Among BRCA1 carriers with a small (<2 cm) node-negative breast cancer, the ten-year survival rate was 91.7% and tumour size was not predictive of survival (HR = 1.01 for 1-2 cm versus 0-1 cm tumors). Chemotherapy was associated with improved survival in BRCA1 carriers (adjusted HR = 0.31; 95% CI 0.10 – 1.00) but not in non-carriers (HR = 1.69; 95% CI 0.95 to 2.99). The interaction between chemotherapy, mutation status and survival was statistically significant (p = 0.009).

Conclusions: The survival of women with breast cancer and a BRCA1 mutation is similar to that of patients without a BRCA1 mutation. For women with a small, node-negative breast cancer and a BRCA1 mutation, the ten-year survival rate was 91.7%. Among women with a BRCA1 mutation, survival was better for women who received chemotheraphy than for women who did not receive chemotherapy. Future studies should investigate what is the optimum chemotherapy regimen.

Acknowledgements: We would like to thank M Siolek, M Szwiec, H Symonowicz, D Surdyka, O Ashuryk, B Gorski, C Cybulski, T Debniak, R Wisniowski and D Sawka for helping to recruit patients to this study.
Material and methods: The studied group comprised 131 patients with urinary bladder carcinoma, diagnosed for the first time and demonstrating various clinical stages (Ta, T1, T2, T3 and T4) and histological grades of malignancy (G1, G2, G3). DNA from tumour cells and DNA, isolated from peripheral blood, were study materials. The obtained DNA was then submitted to an intensive search for CHEK2 (IVS2 + 1G>A gene, 1100delC, del5395, I157T) mutation and for polymorphism of CYP1B1 (355T/T) gene. In order to find out, whether the searched mutations occurred somatically (being not limited to neoplastic cells) or were constitutional in character, the detection was carried out in the DNA from tumour tissue and from peripheral blood. The assumed presence of oncogenic types of HPV was searched in the DNA isolated from epithelial cells in urinary sediment. Seventy-four subjects from control group II were tested for the presence of oncogenic HPV in DNA isolated from epithelial cells in urinary sediment. The control group included 131 patients (control group I, II), in whom tests were run for identification of CHEK2 (IVS2 + 1G>A gene, 1100delC, del5395, I157T) mutation and of CYP1B1 (355T/T) gene polymorphism and oncogenic types of HPV in DNA isolated from epithelial cells in urinary sediment.

Results: In the study group, a total of 11 mutations of CHEK2 gene mutations were identified, while 355T/T polymorphism of CYP1B1 gene was found in 18 cases of the study group (12.9%). In 36 cases (29.3%), out of 123 examined subjects, the presence of an oncogenic HPV type was found. In the control groups I and II, one I157T missense mutation of CHEK2 gene was detected. In both control groups, 355T/T polymorphism of CYP1B1 gene was found in 7 cases. A study was carried out for the presence of oncogenic types of the virus HPV 72 subjects of control group II with indication to HPV infection diagnostics, demonstrating the presence of oncogenic HPV in 32 (44.4%) cases. Seventy-four subjects from control group III with no indications to tests for the presence of oncogenic HPV, constituted a reference group. The presence of the virus was identified in 8 (10.81%) cases.

Conclusions: The performed studies demonstrated a statistically significant difference between the study group and the control group in the incidence of CHEK2 gene mutations, 355T/T polymorphism of CYP1B1 gene and the presence of oncogenic HPV types. Taking into account the obtained results, the following conclusions have been drawn:
1. CHEK2 gene mutations, 355T/T polymorphism of CYP1B1 gene and the presence of oncogenic HPV types are observed with a higher, statistically significant prevalence in neoplastic tissue of urinary bladder carcinoma.
2. The concomitance of CHEK2 gene mutations or 355T/T polymorphism of CYP1B1 gene and of the presence oncogenic HPV types statistically significantly correlates with histological malignancy grades of urinary bladder carcinoma.
3. It seems that occurrence of the mutation of CHEK2 gene, of polymorphism of CYP1B1 gene and of the oncogenic HPV types can be added to the list of genetic and environmental factors, predisposing to urinary bladder carcinoma development and modifying the course of the disease. The amplicons of individual patients were labeled in PCR with MID identifiers and sequenced using a medium-scale next generation sequencing system, GS Junior (Roche/454). The method developed can accurately identify low-level mutations, down to a level of 5% of cells within the testing sample. The lack of progress in head and neck oncology emphasizes the importance of molecular genetic studies to define alterations that may correlate with tumor behavior. Further studies may help clarify this issue.

A22 Overview of genetic markers for hereditary colorectal cancer
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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 4):A22

A number of hereditary conditions have been identified that predispose to colorectal cancer. Most inherited forms of colorectal cancer can be placed into two groups, those that are associated with a pre-malignant phenotype (the “polyposis” syndromes) and those do not have a pre-malignant phenotype (generally termed “non-polyposis”). The polyposis syndromes can be further subdivided into two groups; the hamartomatous polyposis syndromes that include Peutz-Jeghers syndrome (PJS), Cowden’s syndrome (PTEN), juvenile polyposis (JPS) and the adenomatous polyposis syndromes that include familial adenomatous polyposis, Gardner’s syndrome, Oldfield’s syndrome Turcot syndrome, Caroli’s Disease and MUTYH associated polyposis. The non-polyposis group comprises Lynch syndrome, Muir-Torre’s syndrome and Turcot syndrome (there are two forms of Turcot syndrome, one relating to familial adenomatous polyposis and the other to Lynch syndrome).

The genetic basis of the syndromes listed has revealed disease heterogeneity in the non-polyposis group, where all these syndromes are associated with errors in DNA mismatch repair genes (MSH2, MLH1, MSH6 and PMS2). With respect to the adenomatous polyposis syndromes most are associated with mutations in the APC gene, the exception being MUTYH associated polyposis which has been linked to mutations in MUTYH. There are several genes that have been linked to the hamartomatous syndromes, STK11 (LKB1) has been shown to be associated with PJS; PTEN, SMAD4 and BMPR1A with JPS and RMM2 and JPS.

Variance in disease expression can not be accounted solely by mutations in the respective gene. Differences between and within families harbouring the same mutation suggest that other factors are likely to influence disease risk. In the case of Lynch syndrome it is becoming clear that a series of modifier genes are linked to the age of disease onset and further, the number of modifier risk alleles appears to increase disease severity. Recent evidence suggests that two modifiers of particular interest, located on chromosomes 8 and 11 significantly influence disease onset in MLH1 mutation carriers.

Knowledge about modifier genes and how they impact on disease expression is important for improving personalized patient care, especially...
in the setting of life-long surveillance and the potential use of significant prophylactic measures to reduce disease risk.

A23
Diagnostic microarray test for a genetic predisposition to hereditary nonpolyposis colorectal cancer (HNPPC)
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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 4):A23

Colorectal cancer (CC), due to its frequency and social costs is a subject of extensive screening programs (colonoscopy and new developments) in several countries, including Poland. Relatively common and defined genetic predispositions to different forms of CC make genetic screening an attractive alternative, not only in families with positive CC history. Optimal testing strategy should be acceptable for subjects, high-throughput and cost-effective in mutation detection.

CBDNA Ltd. Company has developed and validated prototype diagnostic microarray test for genetic predisposition to nonpolyposis colorectal cancer (HNPPC). The test includes 169 selected mutations (point mutations and short insertions/deletions) in 5 genes: MLH1, MSH2, MSH6, CHD2 and NOD2. Total of 36 patients with CC, six of them fulfilling modified Amsterdam criteria for HNPPC, and the rest with less evident familial aggregation of cancers were included in the pilot study. Altogether 6 different mutations in 9 patients were identified (25%) within the group. The effectiveness of genetic screening using the microarray method was similar to other molecular, but more expensive and time-consuming methods.

A24
Mutations spectrum in hereditary disorders predisposing to occurrence of intestine polyposis in Poland
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The term polyp refers to any overgrowth of tissue from the surface of mucous membranes. Intestinal polyps grow out of the lining of the small and large bowels. The polyps that arise as a result of proliferative dysplasia are termed as adenomatous polyps or adenomas. They are true neoplastic lesions and are precursors of carcinoma. The hamartomatous polyps are formed as a result of abnormal mucosal maturation. They are non-neoplastic and do not have malignant potential. There are several hereditary diseases that produce large numbers of intestinal polyps. These disorders include: familial adenomatous polyposis of the colon (MIM 175100), familial adenomatous polyposis type 2 (MIM 608456), Lynch’s syndrome (MIM 120435), Peutz-Jeghers syndrome (MIM 175200), Juvenile polyposis syndrome (MIM 174900), PTEN Hamartoma Tumor Syndrome (PHTS) PHTS includes: Bannayan-Riley-Ruvalcaba Syndrome (MIM 153480), Cowden Syndrome (MIM 153480), PTEN-Related Proteus Syndrome, Proteus-Like Syndrome. Here we present spectrum of mutation detected in over six hundred Polish families with intestinal polyposis. The studies have encompassed over 30 families with Juvenile polyposis syndrome, over 40 families with Peutz-Jeghers syndrome and almost 600 families with familial adenomatous polyposis of the colon. The study was in part financed by the Ministry of Education and Science, Poland, grant number N402 481537, N401 331936.

A25
KRAS mutation in relation to HER2 overexpression/amplification in colorectal cancer
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The development of targeted therapies for KRAS, EGFR and HER2 may increase the range of response in patients with colorectal cancer. Mutation at codons 12 and 13 of the KRAS gene has been shown to be predictive of cetuximab response in colorectal cancer. However, due to the combined effects of multiple oncogenes involved in disease progression of patients with colorectal cancer, it seems to be important to identify the molecular factors that characterize therapy-resistance phenotype of tumors.

Fifty six paraffin-embedded colorectal cancer specimens were analyzed for KRAS mutation and HER2 overexpression/amplification. A high–resolution melting (HRM) assay and single-nucleotide polymorphisms (SNPs) were used to detect somatic mutation in exon 2 notably codons 12 and 13 of the KRAS gene. HER2 overexpression was detected using monoclonal antibody and confirmed by fluorescence in situ hybridization (FISH) analysis.

KRAS mutations for codons 12 and 13 were identified in 16/46 (34.7%) of patients by SNP. The alterations in KRAS gene were observed in similar percentage of both codons. Colorectal cancer showed mainly heterozygous 35G>A and 38G>A KRAS gene mutations.

HRM analysis showed presence of KRAS exon 2 mutation in 13/46 (28.2%) colorectal cancers. Despite positive SNP results in three cases , HRM technique did not reveal KRAS gene alteration. The concordance rate between the two methodologies was high at 87.5%. KRAS mutation was more frequently observed in poorly differentiated tumors and adenocarcinomas than in other histological types.

HER2 overexpression was found in 37/46 (80.3%) of all colorectal cancers and in 62.1%, 61.5% of KRAS mutation-positive cases detected by SNP and HRM techniques respectively. HER2 overexpression was accompanied by amplification of HER2 gene. The subgroup of colorectal cancers with KRAS mutation and HER2 overexpression/amplification was poorly differentiated.

In summary, the presence of HER2 overexpression/amplification in KRAS mutation–positive colorectal cancers suggests a possible role for the use of specific tyrosine kinase inhibitors in the treatment of disease.

A26
Cumulative small effect genetic markers and the detection of advanced colorectal neoplasias by population screening
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With the instigation of population screening strategies to reduce the burden of colorectal cancer, a cost effective approach remains an elusive goal. Genetic markers associated with colorectal cancer have the potential to be used for the early identification of patient groups at elevated risk of disease. The choice of genetic markers that can be used for screening purposes is population specific. In this report we have genotyped 3059 individuals for 13 markers that have been associated with colorectal cancer risk. The participants underwent colonoscopy and controls with clear colonoscopy (1838) were compared to cases with advanced colorectal neoplasia (213). Logistic regression analysis, adjusted for sex and age at colonoscopy, showed that only one of the markers (rs4779584) was significantly associated with the risk of advanced colorectal neoplasia (OR = 1.93; 95% CI = 1.22 – 2.99; p-value = 0.004; sensitivity = 4.7%). For this combination of 7 markers the linear cumulative risk model was statistically significant (p=7.0·10^-7) after adjustment for sex and age at colonoscopy. The identification of such cumulative models could be valuable in better defining a group of persons, within a given population, that are most likely to benefit from screening for colorectal cancer.

A27 Clinical genetics in cancer prevention
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Cancer family history has been known to be one of the main risk factors. Members of high – risk families should be given recommended screenings, which may improve prophylaxis, early diagnosis and treatment. At present time it is possible to identify several genes involved in the hereditary forms of some types of cancers including colorectal and breast/ovarian. Hereditary forms of breast cancer are mostly caused by mutations in such genes as BRCA1/2 and of hereditary non-polyposis colorectal cancer genes in hMLH1, hMSH2 or hMSH6. Previous studies of Lithuanian population concentrated on breast/ovarian cancer families identified three recurrent mutations 4153delA, 5382insC and C61G in BRCA1 gene. Later studies of unselected cases breast and ovarian cancer patients found that 6% of breast and 19% of ovarian cancer carried a founder mutation in BRCA1 gene. The majority of mutation-positive patients did not have a significant family history. 71.4 % of the breast cancer patients and 87.5% of the ovarian. These and others clinical genetic aspects raise a necessity to change view on routine cancer prevention strategy, as mammography screening for breast cancer with BRCA1/2 mutations, screening by IFOBT and colonoscopy for HPCC and it is necessary to change the view on combination of chemotheraphy (including neo-adjuvant) with BRCA1 positive breast cancer (anthracyclics/taxans).

A28 Prostate cancer screening based on genotyping for high risk founder alleles
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Inherited factors contribute to the lifetime risk of prostate cancer. These factors include a positive family history of cancer and a mutation in one of several prostate cancer susceptibility genes. A number of genome-wide association studies (GWAS) have identified over a number of single nucleotide polymorphisms that have been confirmed to be associated with prostate cancer risk. We evaluated whether or not genotyping of 18 different prostate cancer founder alleles in the Polish population is helpful in identifying high-risk individuals and for determining optimal screening regimens. A serum PSA level was measured and a digital rectal examination was performed on 2907 unaffected men aged 40-90. All men were genotyped for three founder alleles in BRCA1 (5382insC, 4153delA, C61G), four alleles in CHEK2 (1100delC, IVS2+1G>A, del5395, I157T), one allele in NBS1 (657del5), one allele in HOXB13 (G84E), and for nine SNPs which have previously been shown to be associated with prostate cancer risk. A founder mutation in CHEK2 (I157T) predicted prostate cancer in unscreened men.

A29 NBS1 Mutation and prognosis of Prostate Cancer
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Inherited factors contribute to the burden of prostate cancer, however the identification of susceptibility genes for prostate cancer has been challenging. To establish the contribution of eight founder alleles in three DNA damage repair genes (BRCA1, CHEK2 and NBS1) to prostate cancer in Poland, and to measure the impact of these variants on survival among patients, 3750 men with prostate cancer and 3956 cancer-free controls were genotyped for 3 founder alleles in BRCA1 (5382insC, 4153delA, C61G), 4 alleles in CHEK2 (1100delC, IVS2+1G>A, del5395, I157T), and 1 allele in NBS1 (657del5). Strong associations were seen for both CHEK2 and NBS1. BRCA1 was not associated with the risk of prostate cancer, NBS1 mutation was associated with poor survival - mortality was significantly worse for carriers of a NBS1 mutation than for non-carriers (HR = 1.85; p = 0.008). We conclude that a founder mutation in NBS1 predisposes to aggressive prostate cancer.

A30 HOXB13 mutations and prostate cancer in Poland
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Recently, HOXB13 has been established as a prostate cancer susceptibility gene in North America, with a relative risk associated with a single missense mutation of about 20. Ewing et al., sequenced over 200 genes in a prostate cancer linkage region at 17q21-22 among 94 probands of prostate cancer families, and found a recurrent mutation in the HOXB13 gene (G84E) in four families. The mutation co-segregated with prostate cancer. The geographical and ethnic extent of this founder allele has not yet been determined. We assayed for the presence of the G84E mutation in 3515 prostate cancer patients and 2604 controls from Poland. The G84E mutation predisposes to prostate cancer in Poland. We expect that the G84E founder mutation might be present in other Slavic populations.