

# Hereditary cancers – prophylactics, diagnosis, treatment

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### Selected abstracts

[1]

#### Genetic Dissection of Familial Colorectal Cancer

Peltomäki P.

Department of Medical Genetics, University of Helsinki, Finland

Among families with clinical presentation of hereditary nonpolyposis colorectal cancer, 30-70% show no germline DNA mismatch repair (MMR) gene mutations. We previously detected 'hidden' MMR gene defects in 42% of such families, leaving the remaining 58% 'truly' mutation negative. Families with no demonstrable germline mutations in MMR genes differ from mutation-positive families in several essential respects, including later age at onset, generally more distal tumour location, and less frequent occurrence of extracolonic cancers (Renkonen et al. 2003), suggesting a different genetic basis for the latter families.

To obtain clues to the nature of cancer susceptibility in families with no MMR gene mutations, tumours from such families were investigated. These were found to display a unique molecular and clinicopathological profile characterized by a lack of genomic instability (MIN or CIN), normal (membranous)  $\beta$ -catenin, and low frequency of TP53 mutations (Abdel-Rahman et al. 2005). These features distinguish MMR gene mutation negative families from both HNPCC families linked to MMR defects and sporadic cases and should facilitate the identification of novel predisposition genes and pathways in such families.

As an alternative and complementary approach, genetic linkage analysis may be used to identify new cancer predisposition genes. Previous linkage and other studies suggest that as yet unidentified, highly or moderately penetrant colorectal cancer susceptibility genes are likely to exist. A collaborative effort for the identification of novel susceptibility genes in Polish families will be discussed.

#### References

1. Renkonen E, Zhang Y, Lohi H, Salovaara R, Abdel-Rahman WM, Nilbert M, Aittomäki K, Järvinen HJ, Mecklin J-P, Lindblom A and Peltomäki P. Altered expression of MLH1, MSH2 and MSH6 in predisposition to hereditary nonpolyposis colorectal cancer. *J Clin Oncol* 2003; 21: 3629-3637.
2. Abdel-Rahman WM, Ollikainen M, Kariola R, Järvinen HJ, Mecklin J-P, Nyström-Lahti M, Knuutila S and Peltomäki P. Comprehensive characterization of HNPCC-related colorectal cancers reveals striking molecular features in families with no germline mismatch repair gene mutations. *Oncogene* 2005; 24: 1542-1551.

[2]

#### Mutations Causing FAP in the Polish Population

Pławski A., Podralska M., Słomski R.

Institute of Human Genetics, Polish Academy of Sciences, Poznań

Familial adenomatous polyposis (FAP) is an autosomal dominant predisposition to initiate numerous polyps in the colon and rectum which develop into carcinoma if left untreated. FAP is caused by inherited or germline mutations in the APC gene. Early recognition of mutation carriers is very important for the medical treatment of persons from the high-risk group. It is estimated that several hundred Polish FAP families will be subjected to genetic testing. The DNA bank for Polish FAP patients at the Institute of Human Genetics in Poznań was established in 1997. FAP diagnoses were performed in cooperating health centres. 620 DNA samples from persons belonging to 240 FAP families were collected. 280 patients were diagnosed with FAP; 215 persons belong to the risk group and 67 persons are excluded from the risk group. The entire APC gene coding sequence was screened for mutations in 220 families. APC gene mutations were identified in 105 Polish FAP families. Twenty-nine of them have not been described before. Seven mutation types recurred two or more times. Recurrent mutations were detected in 52% of diagnosed families. 90 persons without mutations in the APC gene were further examined for occurrence of MYH gene mutations. Two of the most frequent mutations of the MYH gene (Y165C and G382D) found in this gene occurred in a heterozygotic system in 13% of patients. In the investigations of the MYH gene, no other mutations in the coding sequence were recorded. The results indicate that in our group of patients with diagnosed FAP but without mutation in the APC gene, the proportion of the mutation in the MYH gene has a minor impact on preconditioning the disease.

[3]

#### Germline *MSH2* and *MLH1* Mutational Spectrum Including Large Rearrangements in HNPCC Families from Poland (update study)

Kurzawski G.<sup>1</sup>, Suchy J.<sup>1</sup>, Lener M.<sup>1,2</sup>, Kładny J.<sup>3</sup>, Kłujso-Grabowska E.<sup>1,2</sup>, Safranow K.<sup>4</sup>, Jakubowska A.<sup>1</sup>,

Jakubowska K.<sup>4</sup>, Huzarski T.<sup>1</sup>, Byrski T.<sup>1</sup>, Dębniak T.<sup>1</sup>, Cybulski C.<sup>1</sup>, Gronwald J.<sup>1</sup>, Oszurek O.<sup>1</sup>, Oszutowska D.<sup>1</sup>, Gózdź S.<sup>5</sup>, Niepsuj S.<sup>6</sup>, Słomski R.<sup>7</sup>, Pławski A.<sup>7</sup>, Łącka-Wojciechowska A.<sup>8</sup>, Rozmiarek A.<sup>9</sup>, Fiszera-Maliszewska Ł.<sup>10</sup>, Bębenek M.<sup>11</sup>, Sorokin D.<sup>11</sup>, Sęsiadek M.<sup>12</sup>, Stembalska A.<sup>12</sup>, Grzebieniak Z.<sup>13</sup>, Kilar E.<sup>14</sup>, Stawicka M.<sup>15</sup>, Godlewski D.<sup>15</sup>, Richter P.<sup>16</sup>, Brożek I.<sup>17</sup>, Wysocka B.<sup>17</sup>, Limon J.<sup>17</sup>, Jawień A.<sup>18</sup>, Banaszkiwicz Z.<sup>18</sup>, Janiszewska H.<sup>19</sup>, Kowalczyk J.<sup>20</sup>, Czudowska D.<sup>21</sup>, Scott R.J.<sup>22</sup>, Lubiński J.<sup>1</sup>

<sup>1</sup>International Hereditary Cancer Center – Department of Genetics and Pathology, Pomeranian Medical University, Szczecin, Poland; <sup>2</sup>Department of Natural Science, University of Szczecin, Szczecin, Poland; <sup>3</sup>Division of Surgical Oncology, Pomeranian Medical University, Szczecin, Poland; <sup>4</sup>Department of Biochemistry and Chemistry, Pomeranian Medical University, Szczecin, Poland; <sup>5</sup>Regional Oncology Center, Kielce, Poland; <sup>6</sup>Regional Oncology Hospital, Olsztyn, Poland; <sup>7</sup>Institute of Human Genetics, Polish Academy of Sciences, Poznań, Poland; <sup>8</sup>Genetic Counselling Unit, Great Poland Cancer Center, Poznań, Poland; <sup>9</sup>Regional Hospital, Zielona Góra, Poland; <sup>10</sup>Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland; <sup>11</sup>Regional Oncology Center, Wrocław, Poland; <sup>12</sup>Dept. of Genetics, Wrocław Medical University, Poland; <sup>13</sup>II Katedra i Klinika Chirurgii Ogólnej i Onkologicznej Akademii Medycznej, Wrocław, Poland; <sup>14</sup>Department of Chemotherapy, Regional Hospital, Świdnica, Poland; <sup>15</sup>Prophylactic and Epidemiology Center, Poznań, Poland; <sup>16</sup>Department of General and GI Surgery, Jagiellonian University, Kraków, Poland; <sup>17</sup>Department of Biology and Genetics, Medical University of Gdansk, Gdansk, Poland; <sup>18</sup>Department of Surgery, Ludwik Rydygier University Medical School, Bydgoszcz, Poland; <sup>19</sup>Department of Clinical Genetics, Collegium Medicum, Nicolaus Copernicus University, Bydgoszcz, Poland; <sup>20</sup>Department of Pediatric Hematology and Oncology, Medical University of Lublin, Lublin, Poland; <sup>21</sup>Oncology Diagnostic Center, Legnica, Poland; <sup>22</sup>Discipline of Medical Genetics, Faculty of Health, University of Newcastle and Hunter Medical Research Institute, Newcastle, Australia

Germline mutations in the DNA mismatch repair genes *MSH2* and *MLH1* account for a significant proportion of hereditary non-polyposis colorectal cancer (HNPCC) families. One approach by which development of an efficient DNA testing procedure can be implemented is to describe the nature and frequency of common mutations in particular ethnic groups. Two hundred and twenty-six patients from families matching the Amsterdam II diagnostic criteria or suspected HNPCC criteria were screened for *MSH2* and *MLH1* germline mutations. Fifty different pathogenic mutations were found, twenty-five in *MSH2* and twenty-five in *MLH1*. Twenty-seven of these had not previously been described in other populations. Among our 77 families with *MSH2* or *MLH1* mutations, 51 (67.5%) were affected by recurrent mutations including 38 found at least twice in our own series. Two of the most frequent alterations were a substitution of A to T at the splice donor site of intron 5 of *MSH2* and a missense change (A681T) of *MLH1* found in 10 and 8 families, respectively. Among large deletions detected by the MLPA assay, exon 9 deletions in the *MSH2* gene were found in two families. Our results indicate that a screening protocol specific for the Polish population that is limited to the detection of all reported mutations will result in the identification of the majority of changes present in *MLH1* and *MSH2* genes in Polish HNPCC kindreds.

## [4]

### Frequency and Nature of *MSH6* Germline Mutations in Polish Patients with Colorectal, Endometrial and Ovarian Cancers

Suchy J.<sup>1</sup>, Kurzawski G.<sup>1</sup>, Jakubowska K.<sup>2</sup>, Rać M.E.<sup>2</sup>, Safranow K.<sup>2</sup>, Kładny J.<sup>3</sup>, Rzepka-Górska I.<sup>4</sup>, Chosia M.<sup>5</sup>, Czeszyńska B.<sup>5</sup>, Oszurek O.<sup>1</sup>, Scott R.J.<sup>6</sup>, Lubiński J.<sup>1</sup>

<sup>1</sup>International Hereditary Cancer Center – Department of Genetics and Pathology, Pomeranian Medical University, Szczecin, Poland; <sup>2</sup>Department of Biochemistry and Chemistry, Pomeranian Medical University, Szczecin, Poland; <sup>3</sup>Division of Surgical Oncology, Pomeranian Medical University, Szczecin, Poland; <sup>4</sup>Department of Gynecological Surgery and Oncology of Adults and Adolescents, Pomeranian Medical University, Szczecin, Poland; <sup>5</sup>Department of Pathology, Faculty of Medicine, Pomeranian Medical Academy, Szczecin, Poland; <sup>6</sup>Discipline of Medical Genetics, Faculty of Health, University of Newcastle and Hunter Medical Research Institute, Newcastle, Australia

**Aim:** The aim of the study was to describe the frequency and nature of *MSH6* germline mutations in Polish patients with colorectal, endometrial and ovarian cancers.

**Materials and methods:** 489 colorectal cancer (CRC) cases, 153 endometrial cancer (EC) cases and 179 ovarian cancer (OC) cases were studied, using immunohistochemistry (IHC), DNA sequencing, MLPA, DHPLC and ASA-PCR methods.

**Results:** Seven new alterations and one in other population were found. Five of them were detected in families with colorectal, endometrial or ovarian cancer aggregation among relatives, two in patients with multiple primary tumours and one in a patient with early onset colorectal cancer.

**Conclusions:** The *MSH6* gene should be investigated for occurrence of germline abnormalities in probands affected by CRC, EC or OC and tumours of this type diagnosed among their relatives. Additionally, an *MSH6* examination should be considered for probands with multiple primary tumours (one of them is CRC, EC or OC) or for probands with sporadic, early onset CRC. Laboratory analysis should begin with *MSH6* immunohistochemistry. Then, in cases with no detectable protein expression, DHPLC/sequencing analysis of coding regions (starting from exons 4 and 5) should be performed.

## [5]

### CDKN2A Common Variant and Multi-Organ Cancer Risk – a Population-Based Study

Dębniak T.<sup>1</sup>, Scott R.J.<sup>2</sup>, Huzarski T.<sup>1</sup>, Byrski T.<sup>1</sup>, Rozmiarek A.<sup>3</sup>, Dębniak B.<sup>4</sup>, Górski B.<sup>1</sup>, Cybulski C.<sup>1</sup>, Mędrak K.<sup>1</sup>, Mierzejewski M.<sup>1</sup>, Masojć B.<sup>1</sup>, Matyjasik J.<sup>1</sup>, Złowocka E.<sup>1</sup>, Teodorczyk U.<sup>1</sup>, Lener M.<sup>5</sup>, Kłujso-Grabowska E.<sup>5</sup>, Nej-Wołosiak K.<sup>5</sup>, Jaworowska E.<sup>6</sup>, Oszutowska D.<sup>1</sup>, Szymańska A.<sup>1</sup>, Szymańska-Pasternak J.<sup>1</sup>, Castaneda J.<sup>1</sup>, van de Wetering T.<sup>1</sup>, Suchy J.<sup>1</sup>, Kurzawski G.<sup>1</sup>, Oszurek O.<sup>1</sup>, Narod S.<sup>7</sup>, Lubiński J.<sup>1</sup>

<sup>1</sup>Department of Genetics and Pathology, International Hereditary Cancer Center, Pomeranian Medical University, Szczecin, Poland; <sup>2</sup>Discipline of Medical Genetics, Faculty of Health,

University of Newcastle and Hunter Medical Research Institute, Newcastle, Australia; <sup>3</sup>Regional Hospital, Zielona Góra, Poland; <sup>4</sup>Regional Hospital, Gorzów Wielkopolski, Poland; <sup>5</sup>Inter-University Unit of Molecular Biology, University of Szczecin and Pomeranian Medical University, Szczecin, Poland; <sup>6</sup>Chair and Clinic of Otolaryngology and Laryngological Oncology, Pomeranian Medical University, Szczecin, Poland; <sup>7</sup>Centre for Research on Women's Health, University of Toronto, Canada

The population frequencies of the CDKN2A common variants remain undetermined. In Poland there is a common variant of CDKN2A: an alanine to threonine substitution (A148T), which has been detected in other populations. We have recently shown that it is significantly overrepresented among Polish melanoma patients when compared to the 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. A positive association with the A148T variant was observed for lung cancer (OR 2.0,  $p=0.0052$ ). A similar trend, although non-significant after Bonferroni correction, was observed for colorectal cancer (OR 1.5,  $p=0.5499$ ). These results suggest that the A148T variant may be associated with multi-organ cancer risk in the Polish population.

## [6]

### The Incidence of Mutations of Genes BRCA1, NOD2 and CHEK2 in the Łódź Macroregion

Banaszkiewicz M.<sup>1</sup>, Binka-Kowalska A.<sup>1</sup>, Gilewska A.<sup>1</sup>, Constantinou M.<sup>2</sup>, Kałużewski B.<sup>2</sup>

<sup>1</sup>Nonpublic Health Centre GENOS, Łódź, Poland; <sup>2</sup>Department of Medical Genetics, Medical University of Łódź, Łódź, Poland

It is estimated that about 30% of all neoplasms result from a high, genetically determined predisposition. During recent years, a number of genes have been discovered with mutations responsible for an increased risk of neoplasm occurrence. Genes associated with a predisposition to hereditary neoplasms include: BRCA1, BRCA2, Rb1, MSH2, MLH1 and MSH6. In carriers of the above gene mutations, the risk of neoplastic diseases increases up to as high as 90%.

There is also a large group of genes, the constitutional mutations of which also increase the risk of neoplasm occurrence in certain organs. These are, among others: NBS1, NOD2, CHEK2, CYP1B1 and CDKN2A. An occurrence of mutation in any of the above genes elevates the risk of neoplasm in a number of organs, including the breast, the prostate, the colon, the lungs, the larynx, the ovary, the thyroid and the kidney, as well as of malignant melanoma. Having developed a number of diagnostic tests, it is now possible to determine the constitutional status of patients from the risk groups, followed by implementation of appropriate prophylactic action.

Among the patients examined at our Centre, the incidence of BRCA1 gene mutation was 1.5% (3/214), that of CHEK2 (I157T) was 6% (1/18), and that of NOD2 was 10% (6/60).

## [7]

### Preliminary Results of Investigation of NOD2 3020insC Mutation in Women with Breast Cancer

Janiszewska H.<sup>1</sup>, Haus O.<sup>1</sup>, Lauda-Świeciak A.<sup>1</sup>, Bąk A.<sup>1</sup>, Mierzwa T.<sup>2</sup>, Sir J.<sup>2</sup>, Laskowski R.<sup>2</sup>

<sup>1</sup>Department of Clinical Genetics, Collegium Medicum, Nicolaus Copernicus University, Bydgoszcz, Poland; <sup>2</sup>Oncology Center, Bydgoszcz, Poland

The frameshift NOD2 gene 3020insC mutation is associated especially with Crohn's disease, but predisposes also to many types of common cancers. We studied the frequency of the mutant allele in 148 breast cancer women from the Bydgoszcz region of Poland. The NOD2 mutation was present in 8.8% of patients. The mean age of mutation carriers at breast cancer diagnosis was 43 years. We did not find any mutation in patients diagnosed with breast cancer after the age of 50. There was no association of NOD2 mutation with family breast cancer history. On the contrary, the frequency of mutation (11.4%) was two times higher in the women from families with one case of breast cancer and with aggregation of other common types of cancer, especially digestive tract cancers. Low risk of breast cancer in the mutation carriers seems to be confirmed by the finding of the 3020insC mutation in three healthy parents of probands aged 73, 74 and 83 years, from three separate families.

## [8]

### BRCA1-Positive Breast Cancers in Young Women in Poland

Lubiński J.<sup>1</sup>, Górski B.<sup>1</sup>, Huzarski T.<sup>1</sup>, Byrski T.<sup>1</sup>, Gronwald J.<sup>1</sup>, Domagała W.<sup>2</sup>, Chosia M.<sup>2</sup>, Grzybowska E.<sup>3</sup>, Lange D.<sup>3</sup>, Mika B.<sup>3</sup>, Mackiewicz A.<sup>4</sup>, Karczewska A.<sup>4</sup>, Lamperska K.<sup>4</sup>, Stawicka M.<sup>5</sup>, Bręborowicz G.<sup>6</sup>, Gozdecka-Grodecka S.<sup>4</sup>, Bębenek M.<sup>7</sup>, Sorokin D.<sup>7</sup>, Wojnar A.<sup>7</sup>, Haus O.<sup>8</sup>, Sir J.<sup>8</sup>, Mierzwa T.<sup>9</sup>, Niepsuj S.<sup>10</sup>, Gugąła K.<sup>10</sup>, Góźdz S.<sup>11</sup>, Sygút J.<sup>11</sup>, Kozak-Klonowska B.<sup>11</sup>, Musiatowicz B.<sup>12</sup>, Posmyk M.<sup>12</sup>, Kordek R.<sup>13</sup>, Morawiec Z.<sup>13</sup>, Zambrano O.<sup>13</sup>, Waśko B.<sup>14</sup>, Fudali L.<sup>14</sup>, Surdyka D.<sup>15</sup>, Urbański K.<sup>16</sup>, Mituś J.<sup>16</sup>, Rys J.<sup>16</sup>, Szwiec M.<sup>10</sup>, Rozmarek A.<sup>17</sup>, Dziuba I.<sup>18</sup>, Szczylik C.<sup>19</sup>, Kozak A.<sup>19</sup>, Kozłowski A.<sup>19</sup>, Narod S.A.<sup>20</sup>

<sup>1</sup>Department of Genetics and Pathology, International Hereditary Cancer Center, Pomeranian Medical University, Szczecin, Poland; <sup>2</sup>Department of Pathology, Pomeranian Medical University, Szczecin, Poland; <sup>3</sup>Oncology Center, Gliwice, Poland; <sup>4</sup>Poznań Medical University, Poznań, Poland; <sup>5</sup>Prophylactic and Epidemiology Center, Poznań, Poland; <sup>6</sup>Regional Oncology Center, Poznań, Poland; <sup>7</sup>Regional Oncology Center, Wrocław, Poland; <sup>8</sup>Department of Clinical Genetics, Bydgoszcz Medical University, Poland; <sup>9</sup>Regional Oncology Hospital, Bydgoszcz, Poland; <sup>10</sup>Regional Oncology Hospital, Olsztyn, Poland; <sup>11</sup>Regional Oncology Center, Kielce, Poland; <sup>12</sup>Regional Oncology Center, Białystok, Poland; <sup>13</sup>Department of Chemotherapy, Medical University, Łódź, Poland; <sup>14</sup>Regional Hospital, Rzeszów, Poland; <sup>15</sup>Department of Gynecology, Medical Academy, Lublin, Poland; <sup>16</sup>Regional Oncology Center, Kraków, Poland; <sup>17</sup>Regional Oncology Hospital, Zielona Góra, Poland; <sup>18</sup>Regional Hospital, Koszalin, Poland; <sup>19</sup>Military Hospital, Warsaw, Poland; <sup>20</sup>Centre for Research in Women's Health, University of Toronto, Canada

We identified 4,315 unselected incident cases of early-onset breast cancer (<51 years of age at diagnosis) in 18 Polish

hospitals between 1996 and 2003. We were able to obtain a blood sample for DNA analysis from 3,473 of these (80.5%). All cases were tested for the presence of three founder mutations in BRCA1. The proportion of cases with a BRCA1 mutation was 5.7%. Of the 198 hereditary cases, 36.4% were described as either medullary or atypical medullary and 44.5% were ductal. The tumours in women with BRCA1 mutations were slightly larger, on average, than non-hereditary tumours (2.4 cm versus 2.2 cm;  $p=0.1$ ) but were less likely to be node-positive at diagnosis (35.6% versus 39.6%;  $p=0.04$ ). 57.1% of the hereditary cases had a family history of breast or ovarian cancer.

A significant proportion of breast cancer cases in women diagnosed in Poland under the age of 50 years are due to BRCA1 mutations. These cases could not be reliably identified by family history alone. The association between medullary breast cancer and the presence of a BRCA1 mutation may be greater than previously thought, and emphasizes the importance of recording lymphocytic infiltrates. It is reasonable to offer genetic testing to women with early-onset breast cancer in Poland and to their family members.

## [9]

### Tamoxifen and Contralateral Breast Cancer in BRCA1 and BRCA2 Carriers: an Update

Gronwald J.<sup>1</sup>, Tung N.<sup>2</sup>, Foulkes W.D.<sup>3</sup>, Offit K.<sup>4</sup>, Gershoni R.<sup>5</sup>, Daly M.<sup>6</sup>, Kim-Sing Ch.<sup>7</sup>, Olsson H.<sup>8</sup>, Ainsworth P.<sup>9</sup>, Eisen A.<sup>10</sup>, Saal H.<sup>11</sup>, Friedman E.<sup>12</sup>, Olopade O.<sup>13</sup>, Osborne M.<sup>14</sup>, Weitzel J.<sup>15</sup>, Lynch H.T.<sup>16</sup>, Ghadirian P.<sup>17</sup>, Lubiński J.<sup>1</sup>, Sun P.<sup>18</sup>, Narod S.A.<sup>18</sup> and the Hereditary Breast Cancer Clinical Study Group

<sup>1</sup>International Hereditary Cancer Center, Department of Genetics and Pathology, Pomeranian Medical Academy, Szczecin, Poland; <sup>2</sup>Beth Israel Deaconess Medical Centre, Boston MA; <sup>3</sup>Division of Medical Genetics, Montreal General Hospital, Montreal QC; <sup>4</sup>Department of Human Genetics and Medicine, Memorial Sloan Kettering Cancer Centre, New York NY; <sup>5</sup>Rambam Medical Centre, Haifa, Israel; <sup>6</sup>Fox Chase Cancer Centre, Cheltenham PA; <sup>7</sup>Vancouver Centre, BC Cancer Agency, Vancouver BC; <sup>8</sup>Jubileum Institute, Department of Oncology, Lund University Hospital, Lund Sweden; <sup>9</sup>London Health Sciences Centre, London ON; <sup>10</sup>Department of Medical Oncology, Sunnybrook & Women's College Health Sciences Centre, Toronto ON; <sup>11</sup>Clinical Genetics, Children's Hospital Medical Centre, Cincinnati OH; <sup>12</sup>Oncogenetics Unit, Chaim Sheba Medical Centre, Tel-Hashomer, Israel; <sup>13</sup>Centre for Clinical Cancer Genetics, University of Chicago, Chicago IL; <sup>14</sup>Strang Cancer Prevention Centre, New York NY; <sup>15</sup>City of Hope National Medical Centre, Duarte CA; <sup>16</sup>Department of Preventive Medicine and Public Health, Creighton University School of Medicine, Omaha NE; <sup>17</sup>Epidemiology Research Unit, Centre Hospitalier de l'Université de Montréal (CHUM), Hotel-Dieu, University of Montreal, Montreal QC; <sup>18</sup>Centre for Research in Women's Health, University of Toronto, Toronto ON, Canada

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Women with a mutation in BRCA1 or BRCA2 face a lifetime risk of breast cancer of approximately 80%, and following the first diagnosis the ten-year risk of contralateral breast cancer is approximately 30%. It has been shown that both tamoxifen and oophorectomy prevent contralateral breast cancer, but it is not

clear if there is a benefit to giving tamoxifen to women who have previously undergone an oophorectomy. Furthermore, the relative degree of protection in BRCA1 and BRCA2 carriers has not been well evaluated. We studied 285 women with bilateral breast cancer and a BRCA1 or BRCA2 mutation, and 751 control women with unilateral breast cancer and a BRCA1 or BRCA2 mutation in a matched case-control study. Control women were of similar age and had a similar age of diagnosis of breast cancer and were followed for as long as the case for a second primary breast cancer. The history of tamoxifen use for the first breast cancer was compared between bilateral and unilateral cases. The multivariate odds ratio for contralateral breast cancer associated with tamoxifen use was 0.50 for carriers of BRCA1 mutations (95% CI: 0.30 to 0.85) and was 0.42 for carriers of BRCA2 mutations (95% CI: 0.17 to 1.02). The protective effect of tamoxifen was not seen among women who had undergone an oophorectomy (OR=0.83; 95% CI 0.24 to 2.89) but this subgroup was small. In contrast, a strong protective effect of tamoxifen was apparent among women who had undergone a natural menopause (OR=0.44; 95% CI 0.27 to 0.65).

## [10]

### Paclitaxel and Apoptotic Gene Expression in Breast Cancer Cells

Rzymowska J.<sup>1</sup>, Niemczyk M.<sup>1</sup>, Maj P.<sup>2</sup>, Grzybowska-Szatkowska L.<sup>3</sup>

<sup>1</sup>Department of Biology and Genetics, Medical University, Lublin, Poland; <sup>2</sup>Department of Nuclear Medicine, Medical University, Lublin, Poland; <sup>3</sup>Department of Oncology, Medical University, Lublin, Poland

The aim of this work was to estimate the influence of paclitaxel on activation and inactivation of different genes concerned with development of breast cancer by using the matrix technique. Expression of the genes was calculated in cultures of breast cancer cells which were put under the influence of paclitaxel in 60 ng/ml (PI) and 300 ng/ml (PII) doses in comparison to control cells (K). This made it possible to assess the influence of this drug on the metabolic cycle of the cells. It was thus possible to estimate the activity of different families of genes which participate in carcinogenesis.

A significant increase of caspase gene expression was observed in group PI in comparison to the control group ( $p<0.001$ ). In group PII no significant differences were observed in comparison to group K ( $p>0.05$ ). A significant increase of activity of antiapoptotic genes was observed in group PI in comparison to group K ( $p<0.0001$ ). In the second group a significant statistically weak decrease in expression was observed in comparison to the control group ( $p<0.05$ ). All comparisons between the second and first group concerning caspases and apoptotic genes were significantly higher ( $p<0.0001$ ).

One of the most important processes in stopping development of breast cancer is apoptosis. One of them is anti-apoptosis genes. In this group there are BCL-2 family as BCL-2, BCL-XL, BCLW, MCL, BFL-1, BCL-G). In the second we can distinguish genes which accelerate cell death (BAX, BCLX, BAK, BOK, BAD, BIK, BID, BIM, KRK, MTD, NIP3, BCL-B). Proapoptotic genes such as BCL-X are bound with APAF-1 factor. This process inactivates the release of caspases. In this mechanism the main role take play changes in keeping of proteins by mitochondria. In the case of increasing apoptotic factors the

permeability of the mitochondrial membrane is elevated. This causes release of APAF-1 and activation of 9 and 3 caspases and leads to digestion proteins which are important in living of the cell/cell metabolism. 8 and 10 caspases can be activated by joining TNF to surface membrane receptors. They can also be activated by an external signal carried by such receptors as APO1, IGF1R and TNFR1(2). Signals from these receptors can activate caspases. TP-53 protein, which causes an increase of products taking part in the apoptosis process, participates in this process. It also leads to the release of cyclin c.

In the assessment the activities of two groups of genes concerned with inhibition of apoptosis (BCL2A1, BID, CRADD, DAD1, BECN1, NOL-3, FADD, DNAI A3, CASP1, and caspase (caspase 1-14) were compared.

Table 1.

Genes expression	Control group	Group PI	Group PII
apoptotic genes	14.9±12.9	34.2±2.6**	11.75±4.9*
caspases	11.5± 3.9	31.1±17.1**	9.85±2.03

\*p<0.05 \*\*p<0.001

A significant decrease of anti-apoptotic genes was observed, especially in the second group where levels of paclitaxel were very high. This indicates that paclitaxel inhibits apoptosis processes. The study indicates that in the second group where the level of paclitaxel was five times higher this phenomenon is obvious. Very high levels of paclitaxel are harmful for the cells and cause considerable damage and necrosis.

It seems obvious that such lowering of apoptotic activity in group PII should cause a significant decrease of caspases. It can be explained by a mechanism of inactivation of caspase activity and apoptotic genes by the drug taxane.

## [11]

### Cancer Risks in First-Degree Relatives of BRCA1 Mutation Carriers: Effects of Mutation and Proband Disease Status

Gronwald J.<sup>1</sup>, Huzarski T.<sup>1</sup>, Byrski T.<sup>1</sup>, Mędrak K.<sup>1</sup>, Menkiszak J.<sup>2</sup>, Sun P.<sup>3</sup>, Lubiński J.<sup>1</sup>, Monteiro A.N.<sup>4</sup>, Narod S.A.<sup>3</sup>

<sup>1</sup>Department of Genetics and Pathology, International Hereditary Cancer Center, Pomeranian Medical University, Szczecin, Poland; <sup>2</sup>Clinic of Gynecological Surgery, Pomeranian Medical University, Szczecin, Poland; <sup>3</sup>Centre for Research in Women's Health, University of Toronto, Canada; <sup>4</sup>Lee Moffitt Cancer Center & Research Institute, Tampa, Florida.

We obtained blood samples and pedigree information from 3,568 unselected cases of early-onset breast cancer, and from 609 unselected patients with ovarian cancer from various hospitals situated throughout Poland. Genetic testing was performed for three founder BRCA1 mutations and mutations were identified in 273 samples (187 with 5382insC, 22 with 4153delA and 64 with C61G). A mutation was present in 4.3% of patients with breast cancer and 12.3% of patients with ovarian cancer. We calculated the risk to age 75 in the first-degree

relatives of carriers using Kaplan-Meier methods. The overall risk of breast cancer to age 75 in the relatives was 33% and the risk of ovarian cancer was 15%. The risk for breast cancer was 42% higher among first-degree relatives of carriers of the C61G missense mutation, compared to other mutations (HR=1.42; p=0.10) and the risk for ovarian cancer was lower than average (OR=0.26; p=0.03). Relatives of women diagnosed with breast cancer had a higher risk of breast cancer than the relatives of women diagnosed with ovarian cancer (OR=1.7; p=0.03). The risks for both breast and ovarian cancer were significantly higher in sisters of probands than in mothers, indicating that penetrance appears to be increasing with time.

## [12]

### BRCA1 Mutations and Prostate Cancer in Poland

Cybulski C.<sup>1</sup>, Górski B.<sup>1</sup>, Gliniewicz B.<sup>2</sup>, Sikorski A.<sup>2</sup>, Huzarski T.<sup>1</sup>, Dębniak T.<sup>1</sup>, Jakubowska A.<sup>1</sup>, Gronwald J.<sup>1</sup>, Narod S.A.<sup>3</sup>, Lubiński J.<sup>1</sup>

<sup>1</sup>International Hereditary Cancer Center, Department of Genetics and Pathology, Pomeranian Medical University, Szczecin, Poland; <sup>2</sup>Clinic of Urology, Pomeranian Medical University, Szczecin, Poland; <sup>3</sup>Centre for Research on Women's Health, Toronto, Ontario, Canada

The evidence that BRCA1 mutation carriers are at an increased prostate cancer risk is mixed and both positive and negative studies have been published. To establish whether inherited variation in BRCA1 influences prostate cancer risk in Poland we genotyped 807 cases of prostate cancer and 4,570 controls for the three founder mutations (C61G, 4153delA and 5382insC). A BRCA1 mutation was seen in 0.9% of cases and 0.5% of controls (OR=1.8; p=0.26). However, 4153delA was many times more common in prostate cancer cases (0.5%) than in controls (0.04%) (OR=11.4; 95% CI 2.1–62; p=0.003). The BRCA1 C61G mutation was found in 0.4% of cases compared to 0.06% in controls (OR=5.7; 95% CI 1.1–28; p=0.07). The 5382insC mutation was not detected in any case, whereas it was seen in 0.4% of controls (p=0.16). None of the seven prostate cancer cases with a mutation carried the 5382insC mutation, compared to 17 of 22 individuals with mutations in the control population (p=0.0005). Our study provides evidence that prostate cancer risks may be different for particular alleles of BRCA1.

## [13]

### Founder Mutations in the BRCA1 Gene in Women with Ovarian or Endometrial Cancer

Magnowski P.<sup>1</sup>, Stawicka M.<sup>2</sup>, Górski B.<sup>3</sup>, Lubiński J.<sup>3</sup>, Spaczyński M.<sup>1</sup>

<sup>1</sup>Klinika Onkologii Ginekologicznej, Katedra Ginekologii i Położnictwa, Akademia Medyczna, Poznań, Poland; <sup>2</sup>Onkologiczna Poradnia Genetyczna, OPEN, Poznań, Poland; <sup>3</sup>Ośrodek Nowotworów Dziedzicznych, Zakład Genetyki i Patomorfologii, Pomorska Akademia Medyczna, Szczecin, Poland

The aim of our study was to estimate the frequency of BRCA1 gene founder mutations in the Polish population. The study group

consisted of 106 consecutive patients affected by ovarian cancer and 94 with endometrial cancer. The patients have been treated since 2003 in the Division of Gynecological Oncology at Karol Marcinkowski University of Medical Sciences in Poznań. We have identified BRCA1 gene mutations in 14 of 106 (13.21%) consecutive patients suffering from ovarian cancer. In the group of women with endometrial cancer we found the BRCA1 mutation in one patient. Based on the pedigree-clinical criteria in the group with ovarian cancer we recognised hereditary breast-ovarian cancer syndrome in 22 women, hereditary ovarian cancer syndrome in 10 women and Lynch syndrome in 5 patients. In the group of patients affected by endometrial cancer we found Lynch syndrome in 4 patients (4.2%).

## [14]

### Modulation of Cancer Risk by ESR $\alpha$ and $\beta$ Genetic Polymorphisms in Hereditary Breast and Ovarian Cancer Families

Pękala W.<sup>1</sup>, Polańska J.<sup>2</sup>, Pamuła J.<sup>1</sup>, Grzybowska E.<sup>1</sup>

<sup>1</sup>Department of Tumor Biology, Centre of Oncology, Maria Skłodowska-Curie Memorial Institute, Gliwice, Poland; <sup>2</sup>Institute of Automatic Control, The Silesian University of Technology, Gliwice, Poland

Oestrogen influences the growth, differentiation, and function of breast and several other target tissues by the oestrogen receptors (ESR). ESR $\alpha$  and ESR $\beta$  are the most important mediators of the response to stimulation by oestrogens in target tissues. The mitogenic activity of oestrogens has profound importance in the aetiology and progression of human breast and gynaecological cancers.

The aim of this study was to investigate the possible modulating effect of the polymorphisms in ESR $\alpha$  and ESR $\beta$  genes on the risk of developing malignancy in HBOC patients. The analysis was performed in subjects with HBOC syndrome, divided into two groups:

1. BRCA1/2 – where germline mutation in the BRCA1 or BRCA2 gene was found
2. BRCAx – subjects with HBOC syndrome but without mutation in BRCA1/2  
– versus a control group of healthy women.

We tested 301 persons for PvuII polymorphism in the ESR $\alpha$  gene and AluI polymorphism in the ESR $\beta$  gene. The TA repeat in the ESR $\alpha$  gene was analysed in 283 women and 289 women were examined for CA repeat located in the ESR $\beta$  gene.

There were statistically significant differences in the age of diagnosis between BRCA1/2 and BRCAx groups. The patients with germline mutations in BRCA genes were younger. The diagnoses also significantly differed between the two groups analysed. Carriers of germline mutations in BRCA genes less frequently developed breast cancer and were more frequently diagnosed with ovarian cancer.

The SNPs polymorphisms (Pvu II, AluI) in both genes did not modify the risk of developing cancers in the groups under study.

The length of TA repeats in the ESR $\alpha$  gene showed a strong association with the risk of malignancy in HBOC families. Carriers of long/long genotypes were the most frequent in the population under study so the long/long genotype was chosen as the reference one. Genotype long/short conferred an increased risk

of malignancy, OR=2.44, 95% CI 1.28-4.62  $p=0.00985$ . Much higher risk was connected with the short/short genotype, OR=6.02, 95% CI 2.10-17.19,  $p=0.00095$ . The same trend was observed in the BRCAx group. OR=2.75, 95% CI 1.44-5.26  $p=0.00339$  was calculated for the long/short genotype and also higher risk was found for the short/short genotype, OR=4.60, 95% CI 1.75-12.14,  $p=0.000300$

The length of CA repeats in the ESR $\beta$  gene was not statistically significant except for genotypes long/short and short/short in the BRCAx group. They were a bit more protective against malignancy than in the BRCA1/2 group; OR=0.65, 95% CI 0.35-1.22, and OR=0.60, 95% CI 0.35-1.22, respectively were not statistically significant but OR=0.64, 95% CI 0.36-1.14 for both genotypes was statistically significant ( $p=0.04375$ ).

These results suggest that dinucleotide repeats in introns of ESR $\alpha$  and ESR $\beta$  genes could be associated with modulation of breast and ovary risk in hereditary breast and ovarian cancer families.

## [15]

### The 3' Untranslated Region C>T Polymorphism of Prohibitin is a Breast Cancer Risk Modifier in Polish Women Carrying a BRCA1 Mutation

Jakubowska A.<sup>1</sup>, Gronwald J.<sup>1</sup>, Górski B.<sup>1</sup>, Huzarski T.<sup>1</sup>, Byrski T.<sup>1</sup>, Benner A.<sup>2</sup>, Lubiński J.<sup>1</sup>, Scott R.J.<sup>3</sup>, Hamann U.<sup>2</sup>

<sup>1</sup>International Hereditary Cancer Center – Department of Genetics and Pathology, Pomeranian Medical University, Szczecin, Poland; <sup>2</sup>German Cancer Research Centre, Division of Molecular Genome Analysis, Heidelberg, Germany; <sup>3</sup>Central Unit Biostatistics, Heidelberg, Germany; <sup>3</sup>Discipline of Medical Genetics, School of Biomedical Sciences, University of Newcastle, Newcastle and Hunter Medical Research Institute, Australia

The variable penetrance of breast cancer in BRCA1 mutation carriers suggests that other genetic or environmental factors modify breast cancer risk. The C to T transition in the 3' untranslated region of the prohibitin (PHB) gene alters mRNA function and has been shown to be associated with an increased breast cancer risk among young North-American women who have one first-degree relative with breast cancer.

To investigate whether the PHB 3' UTR polymorphism acts as a modifier of hereditary breast cancer risk we performed a case-control study among female BRCA1 mutation carriers, which included 258 cases and 258 unaffected controls who had both breasts and ovaries intact. Controls were matched to cases by year of birth and BRCA1 mutation (5382insC, 300 T>G, 4153delA).

Genotyping analysis was performed using RFLP-PCR. Odds ratios (OR) were calculated using conditional Maximum Likelihood Estimation for 2x2 tables and Penalized Maximum Likelihood Estimation for logistic regression.

Comparison of frequencies among cases and controls revealed CT (OR, 2.03; 95% CI, 1.17–3.59), TT (OR, 3.17; 95% CI, 0.56–32.25) and combined CT+TT (OR, 2.12; 95% CI, 1.23–3.70) genotypes as significant modifiers of breast cancer risk. Breast cancer risk did not differ between carriers of the 300 T>G and 5382insC mutations. Our results suggest that the PHB 3' UTR T allele increases the incidence of breast cancer in patients who are already at increased risk of disease.

[16]

### Influence of Selected Lifestyle Factors on Breast and Ovarian Cancer Risk in BRCA1 Mutation Carriers from Poland

Gronwald J.<sup>1</sup>, Byrski T.<sup>1</sup>, Huzarski T.<sup>1</sup>, Cybulski C.<sup>1</sup>, Sun P.<sup>2</sup>, Tulman A.<sup>2</sup>, Narod S.A.<sup>2</sup>, Lubiński J.<sup>1</sup>

<sup>1</sup>International Hereditary Cancer Center, Department of Genetics and Pathology, Pomeranian Medical University, Szczecin, Poland; <sup>2</sup>Centre for Research in Women's Health, Sunnybrook and Women's College Health Sciences Center, University of Toronto, Toronto, Ontario, Canada

It has been estimated that the lifetime risk of breast cancer among women who inherit a BRCA1 or BRCA2 mutation is as high as 80%, and the risk estimates for ovarian cancer range from 15 to 40%. Several environmental and lifestyle factors are believed to contribute to the development of breast cancer in the general population and it is of interest to establish if these factors operate among mutation carriers as well. To evaluate the effects of age of menarche, parity, breast-feeding, oophorectomy and oral contraceptive use, as well as smoking and coffee consumption, on the risks of breast and ovarian cancer, we conducted a matched case-control study of Polish women with BRCA1 mutations. There were 348 breast cancer patients, 150 ovarian cancer patients and similar numbers of age-matched controls. BRCA1 carriers with late age of menarche, lower parity and long-term breast-feeding were less likely to develop breast cancer. Oral contraceptives protected against ovarian cancer.

[17]

### A Common Missense Variant in BRCA2 Predisposes to Early Onset Breast Cancer

Górski B.<sup>1</sup>, Narod S.A.<sup>2</sup>, Lubiński J.<sup>1</sup>

<sup>1</sup>Department of Genetics and Pathology, International Hereditary Cancer Center, Pomeranian Medical University, Szczecin, Poland; <sup>2</sup>Centre for Research on Women's Health, University of Toronto, Canada

Mutations in the BRCA2 gene are one of the two major causes of hereditary breast cancer. Protein-truncating mutations of BRCA2 are usually deleterious and increase the risk of breast cancer up to 80% over a lifetime. A few missense mutations in BRCA2 are believed to have a similarly high penetrance, apart from more common neutral polymorphisms. It is often difficult to classify a particular sequence variant as a mutation or a polymorphism. For a deleterious variant, one would expect a greater allele frequency in breast cancer cases than in ethnic-matched controls. In contrast, neutral polymorphic variants should be equally frequent in the two groups.

We genotyped 3,241 cases of breast cancer diagnosed at under 51 years of age, unselected for family history, from 18 hospitals throughout Poland and 2,791 ethnic-matched controls for a single BRCA2 C5972T variant.

The variant was present in approximately 6% of the Polish population. In the study, 13 women (11 cases and two controls (OR=4.7; p=0.02)) were homozygous for the variant allele. The overall odds ratio for breast cancer in women with a single copy of the BRCA2 C5972T variant was 1.1 (p=0.7); however, the effect was significant for patients diagnosed at or before age 40 (OR=1.4; p=0.04). We reviewed the association between

the BRCA2 variant in different histologic subgroups and found the effect most pronounced in women who had ductal carcinoma *in situ* (DCIS) with micro-invasion (OR=2.8; p<0.0001).

The BRCA2 C5972T allele is a common variant in Poland that increases the risk of DCIS with micro-invasion. The homozygous state is rare but increases the risk of breast cancer five-fold.

[18]

### Constitutional Changes in the BRCA2 Gene in Polish Patients with Familial Pancreatic Cancer

Nej-Wołosiak K.

International Hereditary Cancer Center – Department of Genetics and Pathology, Pomeranian Medical University, Szczecin, Poland

Pancreatic cancer is an aggressive and devastating disease, which is characterized by invasiveness, rapid progression and profound resistance to treatment.

According to the data from the year 2000, pancreatic cancer is one of 10 of the most frequent malignancies in the Polish population. Up to now no gene has been identified as specifically predisposing to pancreatic cancer. To gain insight into the possible role of germline BRCA2 mutations in pancreatic cancer, we studied a group of Polish families with aggregation of this tumour.

We report the BRCA2 germline mutation status of 27 families with familial pancreatic cancer. The pancreatic carcinoma families in our study come from the Polish population and did not fulfil the criteria for hereditary breast and ovarian cancer syndrome or any other known tumour syndrome. The median age at diagnosis of pancreatic cancer in these families was 58 years. Using sequencing analysis, we found BRCA2 germline changes: 203 G>A, 353 A>G, IVS 1029+56 C>T, 3199 A>G, 3624 A>G, 3744 G>A, 4035 T>C, 4296 G>A, 4486 G>T, 5427 C>T. Three of them are known as unclassified variants, so the pathogenic roles of these variants are unknown. The rest of the detected changes are considered to be non-pathogenic, because they are synonymous mutations and do not cause protein change.

It could be extremely important to check the frequency of BRCA2 mutations in a larger number of Polish cases of pancreatic cancer patients. Our study could also indicate that further work is necessary to characterize other unknown inherited susceptibility genes in Polish pancreatic cancer patients.

[19]

### High Frequency of Selected Founder Mutations in Russian Breast Cancer Patients

Imyanitov E., Sokolenko A., Iyevleva A., Chekmariova E., Buslov K., Suspitsin E., Togo A., Bit-Sava E., Semiglavov V.

N.N. Petrov Institute of Oncology, St.-Petersburg, Russian Federation

BRCA1 5382insC, CHEK2 1100delC and NBS1 657del5 mutations appear to be relatively common in breast cancer patients originating from Slavic countries. Here we applied a case-control study design in order to evaluate the impact of these variants on

BC incidence in St. Petersburg, Russia. High frequency of the *BRCA1* 5382insC allele was detected in a group of bilateral breast cancer patients (10.4%; 15/144). Randomly selected unilateral BC cases demonstrated noticeable occurrence of the *BRCA1* 5382insC mutation as well (3.7%; 32/857), with an evident excess of carriers in the early-onset ( $\leq 40$  years) category (6.1%; 6/99) and in patients reporting breast and/or ovarian tumours in first-degree relatives (11.3%; 11/97). Strikingly, none of the 478 middle-aged controls and 344 elderly tumour-free women carried the 5382insC variant. *CHEK2* 1100delC demonstrated more modest penetrance, being present in 7/121 (5.8%) bilateral BC, 14/758 (1.8%) unilateral BC, 3/351 (0.9%) healthy donors and 0/344 elderly controls. The corresponding numbers for *NBS1* 657del5 were marginal and approached 2/173 (1.2%), 5/700 (0.7%), 2/348 (0.6%) and 0/348 (0%). The presented data confirm a noticeable contribution of founder mutations in BC development and Russia: in total *BRCA1* 5382insC, *CHEK2* 1100delC and *NBS1* 657del5 variants may explain up to 6% of unselected BC cases and up to 20% of familial-like BC diagnoses.

## [20]

### The 355T Variant of CYP1B1 Predisposes to Breast Cancer in Poland

Matyjasik J.<sup>1</sup>, Cybulski C.<sup>1</sup>, Górski B.<sup>1</sup>, Huzarski T.<sup>1</sup>, Byrski T.<sup>1</sup>, Dębniak T.<sup>1</sup>, Gronwald J.<sup>1</sup>, Masojć B.<sup>1</sup>, Mierzejewski M.<sup>1</sup>, Mędrak K.<sup>1</sup>, Złowocka E.<sup>1</sup>, Suchy J.<sup>1</sup>, Nej-Wołosiak K.<sup>2</sup>, Oszutowska D.<sup>1</sup>, Jaworowska E.<sup>3</sup>, Kowalska E.<sup>1</sup>, Teodorczyk U.<sup>1</sup>, Narod S.A.<sup>4</sup>, Lubiński J.<sup>1</sup>

<sup>1</sup>International Hereditary Cancer Center – Department of Genetics and Pathology, Pomeranian Medical University, Szczecin, Poland; <sup>2</sup>Inter-University Unit of Molecular Biology, University of Szczecin and Pomeranian Medical University, Szczecin, Poland; <sup>3</sup>Department of Otolaryngology and Laryngological Oncology, Szczecin, Poland; <sup>4</sup>Centre for Research on Women's Health, University of Toronto, Canada

CYP1A1 and CYP1B1 are two major enzymes of the cytochrome P450 family and are involved in the hydroxylation of pro-carcinogens to carcinogens. CYP1B1 exceeds CYP1A1 in its catalytic efficiency as a hydrolase, which indicates that CYP1B1 is one of the key players in the carcinogenesis process. The hydroxylation activity of CYP1B1 is of particular importance, because activated carcinogens induce single-strand DNA breaks, thereby increasing the risk of genetic mutation. CYP1B1 metabolises oestradiol to the catechol oestrogen 4-hydroxyestradiol, which has been shown to induce depurination of DNA and lead to DNA mutations. In addition to oestrogens, CYP1B1 bioactivates a range of chemically diverse pro-carcinogens into carcinogens, including benzo[a]pyrene.

Four polymorphisms of the CYP1B1 gene result in amino acid replacement – at codons 48, 119, 432 and 453. Polymorphisms at codons 119 and 432 show high catalytic activity in the region with recognized functional activity – heme-binding region and presumed substrate recognition site 1 (SRS1) of CYP1B1.

We inquired whether the A119S T/T variant of CYP1B1 might predispose to breast and other cancers in Poland. We genotyped 2,033 cases of breast cancer, unselected for family history, from 13 hospitals throughout Poland and 3,353 cases of the control population, consisting of a mixture of 2,056 unselected infants and 1,297 randomly selected individuals. We genotyped cases from an additional 11 other cancer sites: bladder, colon, kidney, larynx, lung, melanoma, ovary, pancreatic, prostate, stomach

and thyroid. The registry for each cancer site contains unselected, histopathologically confirmed cases.

The A119S T/T genotype was present in 11.4% of breast cancer cases and 8.4% of controls (OR=1.4,  $p=0.0005$ ). For seven of the 11 cancer sites the prevalence of the A119S T/T allele exceeded 10%. The excess was statistically significant for three: lung cancer (OR=1.4%; 95% CI: 1.0–1.9), prostate cancer (OR=1.4; 95% CI: 1.0–1.8) and laryngeal cancer (OR=1.5; 95% CI: 1.1–2.2).

In this large multi-site study we show that the CYP1B1 A119S T/T variant appears to be a low penetrance breast cancer susceptibility gene in Poland and it may predispose to cancers of other sites as well. There was suggestive evidence for an effect on the risk of larynx, lung and prostate cancer as well. These associations should be confirmed in other populations.

## [21]

### Association between Early-Onset Breast and Laryngeal Cancers

Jaworowska E.<sup>1</sup>, Masojć B.<sup>2</sup>, Tarnowska C.<sup>1</sup>, Brzosko M.<sup>3</sup>, Fliciński J.<sup>3</sup>, Serrano-Fernandez P.<sup>2</sup>, Matyjasik J.<sup>2</sup>, Amernik K.<sup>1</sup>, Scott R.J.<sup>4</sup>, Lubiński J.<sup>2</sup>

<sup>1</sup>Department of Otolaryngology and Laryngological Oncology, Pomeranian Medical University, Szczecin, Poland; <sup>2</sup>International Hereditary Cancer Center, Department of Genetics and Pathology, Pomeranian Medical University, Szczecin, Poland; <sup>3</sup>Department of Rheumatology, Pomeranian Medical University, Szczecin, Poland; <sup>4</sup>Discipline of Medical Genetics, Faculty of Health, University of Newcastle, and the Hunter Medical Research Institute, Newcastle, NSW Australia

Recent studies suggest that there is a group of genes that predispose simultaneously to both early-onset breast and laryngeal cancer. Studies were performed on a large series of unselected patients with laryngeal cancer diagnosed in Szczecin, Poland. Pedigrees of 683 laryngeal cancer patients were analysed for frequency of early-onset and late-onset breast cancer among first-degree relatives. The observed frequencies of breast cancer in these families were compared to those expected. In addition, common mutations/variants in the three genes *BRCA1*, *NOD2* and *CYP1B1*, known to be associated with early-onset breast cancer, were assessed to determine their frequency in 348 unselected laryngeal cancers. The average age at diagnosis of LC among patients who had relatives affected by BC diagnosed under the age of 50 years was 57.62. In comparison, LC patients reporting a first-degree relative affected by BC diagnosed above 50 years of age had an average age of diagnosis of 66.00 years, which was significantly different ( $p=0.0064$ ). Similarly, the average age of diagnosis of BC among patients with LC diagnosed under the age of 50 years was 46.7 years, whereas LC patients with tumours diagnosed above 50 years had relatives diagnosed with breast cancer at an average age of 53.37 years, which was significantly different ( $p=0.02$ ).

From the 348 consecutive ascertained laryngeal cancer patients who had molecular studies undertaken, breast cancers among first-degree relatives were found in 18 families including 8 with breast cancers diagnosed at less than 50 years of age. A molecular basis was identified (the CYP1B1 355T/T genotype) in only 2 of the 8 early cases, suggestive of there being additional, as yet unknown genes that are associated with an early-onset laryngeal-breast cancer phenotype.

## [22]

**Prevalence of the NOD2 3020insC Mutation in Aggregations of Breast and Lung Cancer**

Lener M.<sup>1,5</sup>, Oszutowska D.<sup>1</sup>, Castaneda J.<sup>1</sup>, Kurzawski G.<sup>1</sup>, Suchy J.<sup>1</sup>, Nej-Wołosiak K.<sup>1,5</sup>, Byrski T.<sup>1</sup>, Huzarski T.<sup>1</sup>, Gronwald J.<sup>1</sup>, Szymańska A.<sup>1</sup>, Szymańska-Pasternak J.<sup>1</sup>, Grodzki T.<sup>2</sup>, Serwatowski P.<sup>2</sup>, Bręborowicz G.<sup>3</sup>, Scott R.J.<sup>4</sup>, Lubiński J.<sup>1</sup>

<sup>1</sup>International Hereditary Cancer Center Department of Genetics and Pathology, Pomeranian Medical University, Szczecin, Poland; <sup>2</sup>Regional Hospital for Lung Diseases, Szczecin-Zdunowo, Poland; <sup>3</sup>University School of Medical Sciences, Department of Perinatology and Gynecology, University Hospital, Poznań, Poland; <sup>4</sup>Discipline of Medical Genetics, Faculty of Health, University of Newcastle and the Hunter Medical Research Institute, New South Wales 2305, Australia; <sup>5</sup>Department of Natural Science, University of Szczecin, Szczecin, Poland

Both breast and lung cancers are common malignancies, and within the context of known genetic predispositions to breast cancer, no association has been made in linking the two diseases together. This does not exclude the possibility that such associations may exist that lie outside the known high-risk breast cancer families. To examine the likelihood of common genetic factors that could influence the risk of disease, two sets of consecutively collected tumour groups were examined for the 3020insC mutation in the NOD2/CARD15 gene. A total of 4,107 consecutively collected breast cancer patients were assessed for prevalence of the 3020insC mutation and compared to a consecutively collected series of 389 lung cancer patients and 2068 control samples. The results revealed that a proportion of breast cancer patients who had a first- or a second-degree relative diagnosed with lung cancer were more likely to harbour a change in NOD2/CARD15 compared to patients who had no relatives affected by lung cancer. Furthermore, this difference appeared to be specific to the breast and lung cancer subgroup since there was no difference in the frequency of the 3020insC allele in the consecutively collected lung cancer patients.

In conclusion, it appears that the 3020insC mutation of the NOD2/CARD15 gene may be a genetic predisposing factor for aggregations of breast and lung cancer.

## [23]

**Mutations of the CHEK2 Gene in Patients with Borderline Cystadenomas of the Ovary**

Szymańska-Pasternak J., Szymańska A., Cybulski C., Górski B., Lubiński J.

International Hereditary Cancer Center, Department of Genetics and Pathology, Pomeranian Medical University, Szczecin, Poland

Borderline tumours are noninvasive neoplasms that have nuclear abnormalities and mitotic activity intermediate between benign and malignant tumours. The most common borderline surface epithelial-stromal lesions of the ovary are serous and mucinous tumors, which may undergo malignant transformation into cystadenocarcinoma.

Recently it was found that CHEK2 is a multiorgan cancer susceptibility gene. Mutations in the CHEK2 gene have been

associated with a predisposition to breast, colon, kidney, prostate and thyroid cancer. We examined borderline cystadenomas for occurrence of three founder alleles characteristic for the Polish population.

The study group comprised 119 female patients with borderline ovarian cystadenoma: 87 with serous type and 32 with mucinous type. Cystadenoma cases were collected from five provinces of Poland between 1997 and 2005. Study subjects were unselected for age and for family history. The control group consisted of 4,000 individuals.

The missense variant of CHEK2 gene was present in 11.8% of women with borderline cystadenomas and 4.8% in controls (OR=2.6; p=0.0014). This positive association was also found for serous cystadenoma of borderline malignancy (OR=3.2; p=0.0004). Relative risk was increased particularly for cystadenomas diagnosed under the age of 50 years (OR=2.7; p=0.006 for all cases and OR=3.04; p=0.0066 for serous subgroup). Heterozygotes among the mucinous subgroup were identified as the most frequent in young patients diagnosed under 40 years, but this excess was not statistically significant (OR=2.8; p=0.3994). We did not find any statistically significant differences for CHEK2-truncating mutations.

The findings of our study suggest that the I157T variant of the CHEK2 gene may predispose to borderline cystadenoma in women at reproductive age.

## [24]

**The Molecular Pathway as a Global Genetic Source of Cancer Risk**

Serrano-Fernández P., Wokołorczyk D., Matyjasik J., Dębniak T., Lubiński J.

International Hereditary Cancer Center, Department of Genetics and Pathology, Pomeranian Medical University, Szczecin, Poland

In recent years several gene mutations have been demonstrated to be associated with an increased risk of developing a particular cancer type. Those findings have mostly been analysed independently. Some genetic risk sources could in fact affect presumably independent genetic networks (e.g. the ones responsible for DNA repair versus the ones responsible for angiogenesis). However, it is reasonable to think that the number of completely independent networks will be limited and many of the mutations contributing to increased cancer risk will be functionally linked in the same pathway.

Known mutations on 7 genes – previously described as associated with breast cancer in the Polish population – were checked for their functional interrelationships and a molecular network was built accordingly. We then analysed the incidence of mutations on that pathway as a whole in breast cancer patients (575 individuals) as compared with controls (1,175 individuals). Moreover we checked for specific dyadic interactions leading to an unexpectedly high increase of cancer risk.

Results significantly show how the cancer risk geometrically grows with the accumulation of mutations in the predefined pathway. Logistic regression showed additionally a significant interaction effect between 2 of the 7 genes.

Considering the whole pathway as a genetic risk source improves the accuracy of epidemiological models since it takes into account interactive models, where the final increase in the cumulative risk does not result just from the addition of the partial

risks associated with each mutation separately. Indirectly, it also offers – for basic research – a quantitative background for unravelling regulatory relationships in a pathway.

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## [25]

### Identification of *NBS1* Gene Molecular Variants in Patients with Paediatric Brain Tumours

Ciara E.<sup>1</sup>, Piekutowska-Abramczuk D.<sup>1</sup>, Popowska E.<sup>1</sup>, Grajkowska W.<sup>2</sup>, Perek D.<sup>3</sup>, Dembowska-Bagińska B.<sup>3</sup>, Roszkowski M.<sup>4</sup>, Barszcz S.<sup>4</sup>, Krajewska-Walasek M.<sup>1</sup>, Chrzanowska K.<sup>1</sup>

<sup>1</sup>Department of Medical Genetics; <sup>2</sup>Department of Pathology; <sup>3</sup>Clinic of Oncology; <sup>4</sup>Clinic of Neurosurgery, The Children's Memorial Health Institute

Mutations in the *NBS1* gene are associated with Nijmegen breakage syndrome (NBS) – autosomal recessive disorder. Nibrin, the product of the *NBS1* gene, is functionally involved in the double strand DNA repair system. Heterozygous carriers of *NBS1* mutations have a strongly increased risk of cancer. The aim of the study was to analyse the mutations and polymorphisms in the *NBS1* gene in children with different tumours. The blood DNA samples (DBS) of 201 patients and the different tumour DNA samples of 68 patients were screened for two of the most common mutations in exon 6 of the *NBS1* gene: c.657del5 and c.643C>T (p.R215W). The molecular study of medulloblastoma DNA samples of 38 patients consisted of analyses of exons 3, 5, 6, 7, 8, 10 and 13. All samples were analysed by PCR-SSCP and by direct sequencing. The four *NBS1* heterozygous mutations were detected in all analysed patients. Among the 233 patients the c.657del5 mutation was identified on one allele in two patients and the c.643C>T (p.R215W) mutation on one allele in one patient. The frequency of the c.657del5 mutation was calculated as 0.0043 and the c.643C>T mutation was 0.0021. The values are similar to the frequency calculated for the mutations in controls (0.0060 and 0.0030, respectively). In the group of 38 patients with medulloblastoma, one heterozygous carrier for the c.511A>G (p.I171V) mutation in exon 5 of the *NBS1* gene was identified. In addition, three frequent sequence variants, c.553A>G (p.E185Q), c.2016A>G (p.P672P), and IVS12-7A>G, were revealed. Larger studies are needed to evaluate the impact of *NBS1* gene heterozygosity in susceptibility to paediatric brain tumours.

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## [26]

### Cancer Familial Aggregation (CFA) and G446A Polymorphism in the *ARLTS1* Gene

Masojć B., Mierzejewski M., Cybulski C., Van de Wetering T., Dębniak T., Górski B., Jaworowska E., Lener M., Scott R.J., Lubiński J.

International Hereditary Cancer Center – Department of Genetics and Pathology, Pomeranian Medical University, Szczecin, Poland

*ARLTS1* – a member of the ADP-ribosylation factor family – is a newly-described candidate tumour suppressor gene. Recent studies show that a nonsense polymorphism, G446A (Trp149Stop), in the *ARLTS1* gene is significantly more frequent in familial cancer cases than in sporadic cancer cases. This study presents an analysis of the germline G446A polymorphism in the *ARLTS1* gene among 1,686 consecutively collected patients with breast cancer, prostate cancer, malignant melanoma, thyroid papillary cancer or laryngeal cancer in Poland. The G446A allele was present in 1.81% (9/497) of breast cancer patients, 1.46% (5/343) of prostate cancer patients, 1.76% (7/397) of melanoma patients, 1.65% (3/182) of thyroid papillary carcinoma patients and 2.68% (8/299) of laryngeal cancer patients. The frequency of this polymorphism in the control group was 1.45% (8/552). Differences in the frequency of the G446A polymorphism between case and control groups were not statistically significant. In addition, there was no significant difference in the number of Cancer Familial Aggregations (CFA) among breast, prostate, thyroid or laryngeal cancer cases harbouring the G446A polymorphism, when compared to the G446A negative cases. Interestingly, out of the CFA melanoma cases, 4/6 (66.6%) were found to harbour the change compared to only 20.2% (69/341) of sporadic melanoma cases. This difference was statistically significant ( $p=0.02$ ,  $OR=7.8$ ). The results of this study suggest that the G446A in *ARLTS1* gene is probably not associated with an increased risk of sporadic breast cancer, prostate cancer, melanoma, thyroid papillary cancer or laryngeal cancer. Moreover, the G446A polymorphism is not significantly more frequent in CFA cases except for families in which the proband had melanoma. To confirm this result more cases of melanoma should be analysed.

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## [27]

### Primary Cutaneous T-Cell Lymphomas Show a Deletion or Translocation Affecting *NAV3*, the Human *UNC-53* Homologue – the Lesson from Chromosome Aberrations for Suppressor Gene Finding

Nedoszytko B.<sup>1</sup>, Karenko L.<sup>7</sup>, Hahtol S.<sup>7</sup>, Päivinen S.<sup>7</sup>, Karhu R.<sup>3,4</sup>, Syrjä S.<sup>2</sup>, Kähkönen M.<sup>5</sup>, Kytölä S.<sup>3</sup>, Zhou Y.<sup>7</sup>, Blazevic V.<sup>6</sup>, Pesonen M.<sup>1</sup>, Nevala H.<sup>1</sup>, Nupponen N.<sup>2</sup>, Sihto H.<sup>2</sup>, Krebs I.<sup>8</sup>, Poustka A.<sup>8</sup>, Roszkiewicz J.<sup>1</sup>, Saksela K.<sup>4</sup>, Peterson P.<sup>4</sup>, Visakorpi T.<sup>3,4</sup>, Ranki A.<sup>7</sup>

<sup>1</sup>Department of Dermatology, Medical University of Gdansk, Gdańsk, Poland; <sup>2,7</sup>Departments of Dermatology and Venereology and Oncology, Helsinki University Central Hospital, University of Helsinki, Helsinki, Finland; <sup>3,4,5</sup>Laboratory of Cancer Genetics, Institute of Medical Technology, and Department of Clinical Genetics, Tampere University Hospital, University of Tampere; <sup>6,7,8</sup>FIT Biotech, Ltd., Tampere, Finland; and Division of Molecular Genome Analysis, German Cancer Research Center, Heidelberg, Germany

Multicolour fluorescent in situ hybridization (FISH) was used to identify acquired chromosomal aberrations in 12 patients with mycosis fungoides or Sezary syndrome, the most common forms of primary cutaneous T-cell lymphoma (CTCL). The most frequently affected chromosome was 12, which showed clonal deletions or translocations with a break point in 12q21 or 12q22 in five of seven consecutive Sezary syndrome patients and a clonal monosomy in the sixth patient. The break point of a balanced translocation t(12;18)(q21;q21.2), mapped in the minimal common region of two deletions, fine mapped to 12q2. By

locus-specific FISH, the translocation disrupted one gene, NAV3 (POMFIL1), a human homologue of *unc-53* in *Caenorhabditis elegans*. A missense mutation in the remaining NAV3 allele was found in one of six cases with a deletion or translocation. With locus-specific FISH, NAV3 deletions were found in the skin lesions of four of eight (50%) patients with early mycosis fungoides (stages IA-IIA) and in the skin or lymph node of 11 of 13 (85%) patients with advanced mycosis fungoides or Sezary syndrome. Preliminary functional studies with lentiviral small interfering RNA-based NAV3 silencing in Jurkat cells and in primary lymphocytes showed enhanced interleukin 2 expression (but not CD25 expression). Thus, NAV3 may contribute to the growth, differentiation, and apoptosis of CTCL cells as well as to the skewing from Th1-type to Th2-type phenotype during disease progression. NAV3, a novel putative haploinsufficient tumour suppressor gene, is disrupted in most cases of the commonest types of CTCL and may thus provide a new diagnostic tool.

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## [28]

### The Challenge of Individualizing Genetic Counselling in Cancer Families – a Case Report

Pesz K., Trusewicz A., Ślęzak R., Sęsiadek M.

Dept. of Genetics, Wrocław Medical University, Wrocław, Poland

Although the pedigree criteria for hereditary breast and ovarian cancer and indications for DNA testing seem to be clearly established from time to time, a snag arises when the pedigree does not reveal a conclusive pattern for cancer inheritance, making the cancer risk assessment difficult. Then the benefit/risk ratio of offering or not offering molecular testing must be taken into account by the genetic counselling provider. To illustrate this point we present a family with a pedigree that seemed disputable as far as BRCA1 testing was concerned. The proband was seeking genetic counselling after losing three of his closest relatives to cancer. Taking the pedigree into consideration the diagnostic algorithm for testing was established. Overall we searched for 10 different mutations in five different genes. Finding BRCA1 mutation carriers in this family enabled us to make the diagnosis of hereditary breast/ovarian cancer syndrome. It can be concluded from the study that each family should be treated individually and genetic counselling provides an opportunity for it.

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## [29]

### Cost Effectiveness Analysis of a Prophylactic, Genetic-Oncological Programme in the West Pomeranian Region – Initial Announcement of Results

Marska N.

Department of Finance, Faculty of Economics and Management, University of Szczecin, Poland

One area of disability policy is prevention, which is described as reducing the risk of entering an undesirable state. Most areas of intervention analysed so far have considered protection and

promotion, and they are well developed and expensive. Because it is difficult to take the awarded rights back and disability assessment is a complicated issue, the best way is to limit the application number. This is why the problem of prevention, and within it also prophylaxis, has become the centre of attention. Furthermore, the problem of social expenses rises especially within prevention programmes, where simple economic models are difficult to implement. After 1989, the supremacy of financial aspects over social ones caused a shortage in epidemiological research.

An example of prophylactic activity in cancer prevention is the Centre of Hereditary Tumours located at Pomeranian Medical University in Szczecin. Since 2003 the author of this paper in cooperation with Prof. Jan Lubiński, the Head of the Centre and the national consultant of clinical genetics, has conducted a research programme titled: Cost effectiveness analysis of a prophylactic, genetic-oncological programme in the West Pomeranian region. The main aim is to identify, evaluate and compare the real costs of a prophylactic programme with the direct and most indirect costs, coming from breast and ovarian cancer. To achieve this it was necessary to identify and calculate the costs of the questionnaire programme conducted during 1999-2003, the costs of the prophylactic programme – ‘to lead off’ the patient during the following years, the real costs of medical treatment, the real costs coming from social insurance benefits paid to patients and the potential costs of temporary or permanent exclusion from the working population. Such kinds of research are being carried out in Poland for the first time. There were some tests taken, though only for direct costing. The research group consisted mainly of patients with breast cancer and a few with breast and ovarian cancer, for which diagnosis has been made in 1999-2000. The social costs were evaluated for the period 2000-2003 (treatment, recovery and rehabilitation).

The medical costs of cancer treatment (breast or breast and ovarian) were around USD 6,500 and were calculated for the full treatment procedure (usually 3 years). The indirect cost – the amount of disability pension and additional benefits received during 4 analysed years – was USD 10,330. The economic cost – the lost GDP – if calculated for the total population equals USD 26,650, and if calculated for the population active on the labour market equals USD 59,112. Conclusively, the total cost amounts to a minimum of USD 43,500, that is USD 10,870 per year on average. Taking into consideration the costs of screening tests, the costs of periodical examinations of patients with BRCA1 or BRCA2, the preventive surgery and the reduction of probability of an illness, the total cost per person equals USD 6,000, but this is spread out over many years.