mutation spectrum might be more dispersed than previously observed. Aberrations within BRCA1/2 promoters, which can result in BRCA1/2 protein decrease, could be also associated with an increased risk of the disease.

Patients and Methods: One hundred and fifty unrelated probands with strong family history of breast and ovarian cancer, bilateral or early-onset breast cancer we screened by direct sequencing of all exons and exon/intron boundaries.The sequence of BRCA1 promoter region were also analysed in 87 breast/ovarian cancer cases without mutation in BRCA1/2 genes.

Results: We found 2 families with deletions in beta-promoter of BRCA1 (No.Ac U37574, 2223delAAAAA) and 5 polymorphisms in BRCA1 promoter region (No.Ac U37574, 2642A>G, 2743T>C, 1895G>C, 1983G>C, 1873G>C). Any important aberrations in this region have not been reported previously. We also found 5 different disease predisposing mutations within BRCA1 gene (185delAG, 300T/G, 4153delA, 5382insC, 5528del1+IV22-6). The results confirms the presence of two strong BRCA1 founder mutations in Polish population-5382insC and 300T>G. The BRCA1 (5528del1+IV22-6) mutation were not reported previously and might be specific to the southern Polish population while the others were recurrent. We also detected two new sequence variants in BRCA1 introns (IVS12-4 and IVS21-31). Two disease predisposing mutations were detected in BRCA2 (6174delT, 9631delC). In addition, eight new unclassified sequence variants were found within BRCA2 exons (3431T>C, 3446A>G, 3655G>C, 4846G>T, 4988C>T, 6188A>G, 8335A>T, 8341A>T) and 6 new variants in BRCA2 introns (IVS4+67, IVS4+147, IVS12+157, IVS12+183, IVS18+13, IVS24-36).

Conclusion: Identified novel aberrations in the BRCA1 promoter suggest that mutation and polymorphisms in this region might be responsible for significant fraction of breast and ovarian cancer cases. Our results lend further support to the need for more detailed functional and epidemiological studies aimed at understanding the role of BRCA1 and BRCA2 promoters in the etiology of breast and cancer.

409 POSTER

Clinical prognosis of BRCA1-associated breast cancer: a cohort study from the upper silesia region in Poland.

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Background: To compare the pathologic characteristics, survival, and the incidence of second cancer in three groups of patients with breast cancer: A) BRCA1 mutation carriers, B) BRCA1 non-carriers, C) patients who never had genetic counseling.

Material/Methods: Women affected with breast cancer were encouraged to attend genetic counseling if they had a family history of breast or ovarian cancer, and/or presented with bilateral breast cancer. Screening for BRCA1 mutations was performed using ASA-PCR. Forty-six carriers of BRCA1 mutation were identified: 5382insC, and 9631delC constituted 82%. From the database of patients who had genetic counseling, but had no identified BRCA1 mutation we matched a control group of 46 patients using a year of the diagnosis as a sole stratification criterion. Likewise, 46 patients who never had genetic counseling were matched to create a second control group.

Results: Patients in groups A and B were younger at the diagnosis than those in group C. Tumour grade was higher in group A than in group B and C. Group C presented at more advanced clinical stage than group A and B. Eighty percent of patients in group A lacked estrogen receptor expression vs. 40% and 30% in group B and C. A high incidence of second breast cancer in group A and B (43% vs. 43%) compared to group C (2%) was attributable to the counseling criteria. The actuarial 10-years metastases-free survival was 82%, 65% and 54% in groups A, B, C respectively. The corresponding actuarial 10-years local recurrence-free survival was 95%, 80% and 75%. Eight patients from group A developed ovarian cancer compared to only 1 and 0 from groups B and C. The overall 10-year actuarial survival was significantly lower in group C (35%), but did not differ significantly in groups A and B (80 vs. 81%).

Conclusions: These data show a low incidence of distant metastases and local recurrences among BRCA1 mutation carriers, in spite of unfavorable pathologic features and high incidence of second breast cancers in this group. However, the overall survival of BRCA1 mutation carriers did not differ significantly from non-carriers due to frequent occurrence of ovarian cancers which eventually relapsed. Patients who never had genetic counseling presented in most advanced stages, and carried the most unfavorable prognosis. This shows that the counseling may carry the bias of artificially increasing survival times by excluding patients with most advanced disease at the diagnosis.

410 POSTER

Correlation between BLC-2 protein expression and the clinical, pathological and biological characteristics of 483 breast cancer patients

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Introduction: Apoptosis, or programmed cell death, plays a critical role in the development of cancer. The BCL-2 oncogene is currently believed to be important in suppressing apoptosis and, among the recently proposed putative prognostic markers of breast cancer, considerable attention has been given to the products of the BCL-2 proto-oncogene.

Patients and methods: We evaluated the immunohistochemical expression of BCL-2 in 483 stage I and II breast cancer patients and assessed its relationship with a number of clinicopathological outcome predictors.

Results: BCL-2 immunoreactivity was observed in 413 cases (85%), being significantly higher in node-negative ($p<0.001$), ER- and PgR-positive ($p<0.001$), slowly proliferating and well-differentiated tumours ($p<0.001$). BCL-2 immunostaining did not correlate with pT, c-erbB2, vascular invasion or age, nor with the other proteins involved in regulating cell death and tumour proliferation, such as p53 and p21. The same analyses were repeated in 69 cases of *in situ* ductal carcinoma, and showed a close correlation between BCL-2 and ER/PgR positivity.

Conclusion: The immunohistochemical expression of BCL-2 protein in breast cancer patients is associated with a prognostically favourable phenotype and seems to be related to hormonal regulation. The prognostic data will be avaliable at the meeting.

411 POSTER

Highly sensitive detection of the MGB1 transcript(mammaglobin) in the peripheral blood of breast cancer patients

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Background: In recent years, several reverse-transcriptase polymerase chain reaction (RT-PCR) assays using epithelial [(e.g. citokeratin 19 (*CK19*), citokeratin 20 (*CK20*)] and supposedly mammary-specific markers [e.g. maspin (*SERPINB5*)] have been developed for the detection of disseminated breast cancer cells, but the sensitivity of these molecular markers is controversial and their specificity limited by the fact that these genes are expressed at low levels in blood cells, a phenomenon known as illegitimate transcription. Recently, the mammaglobin gene (*MGB1*) was described to be a potentially specific marker for the detection of circulating breast tumor cells since his expression was reported to be restricted to the mammary epithelium

Material and Methods: A new One-Step Nested reverse-transcriptase polymerase chain reaction (RT-PCR) assay for the detection of the mammaglobin (*MGB1*) gene transcript in the peripheral blood of breast cancer patients was applied to the study of 54 breast cancer patients. The control group included 38 peripheral blood samples from healthy donors and 18 samples from patients with hematopoietic malignancies.

Results: The *MGB1* transcript could be detected in the peripheral blood of 24 of 54 (41%) breast cancer patients prior to any therapy. Our method, using specific primers for cDNA synthesis, proved to be more sensitive (10^{-6} to 10^{-11}, usually 10^{-7}) than previously reported methodologies. This increased sensitivity was achieved without compromising specificity, as the *MGB1* transcript was not detected in 38 blood samples of healthy donors and in only one of 18 blood samples of patients presenting with hematological malignancies. A positive correlation was seen between *MGB1* positivity and breast cancer stage: 0/3 (0%) in stage 0, 3/13 (23%) in stage I, 6/17 (35%) in stage II, 5/10 (50%) in stage III, and 8/11 (73%) in stage IV ($p=0.003$).

Conclusion: We have found that the proportion of breast cancer patients with *MGB1* peripheral blood positivity increases with disease stage: 0% in stage 0, 23% in stage I, 35% in stage II, 50% in stage III, and 73% in stage IV. Although t he linear trend for *MGB1* positivity according to clinical stage was statistically significant ($p = 0.003$), the prognostic value of this marker, especially in clinically localized disease, must be evaluated after long-term clinical follow-up of these patients.