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CHEK2 I157T is not associated with an increased risk of endometrial cancer in Bulgarian patients

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The CHEK2 gene encodes a cell cycle checkpoint kinase which is crucial for DNA-damage signaling and cell cycle regulation.

Germline variants in CHEK2 gene have been shown to act as low-penetrance cancer susceptibility alleles in a variety of human malignancies. CHEK2 I157T variant has particularly been associated with an increased risk for colon and breast cancer, but at the same time, to have a protective role for lung cancer.

In order to estimate the significance of this polymorphism for endometrial cancer susceptibility, we have genotyped 240 patients and 449 female control subjects in a case-control study. Patients were recruited mainly from North-Western Bulgaria. Controls were anonymous females from throughout the country. We used germline DNA extracted from venous blood to perform PCR-RFLP analysis with Pst I enzyme. Variant carriers were directly sequenced for confirmation. The distribution of genotype frequencies among groups were compared using $\div^2$ test. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated using unconditional logistic regression.

CHEK2 I157T variant was more often found among controls – in 2.45 % (11 of 449), than among patients – 1.67 %, where we found 4 carriers in 240 patients (OR, 0.67; 95% CI, 0.18 – 2.32). We did not identify homozygous variant carriers in both groups. The estimated frequency of the variant in healthy Bulgarian controls is lower than that found in northern and central European populations.

These results suggest that CHEK2 I157T does not increase endometrial cancer susceptibility. Further studies on a larger scale may contribute to understanding the role of CHEK2 I157T for endometrial cancerogenesis.

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Mutation screening of BRCA1 exon 11 in Bulgarian breast cancer families

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Background: Studies on different populations worldwide demonstrate that germ line mutations in BRCA1 and BRCA2 cancer susceptible genes account for the majority of hereditary breast and ovarian cancers.

Materials and methods: We have screened 51 high-risk breast cancer families from Bulgaria for mutations and polymorphisms in exon 11 of BRCA1. Mutation analysis was performed by direct sequencing using 12 primer pairs covering exon 11, which composes about 70% of the total coding sequence of the gene.

Results: One frequent variant (25.5%), a missense mutation Q356R at nucleotide positions 1186, was detected in exon 11 of BRCA1gene among studied breast cancer families. This variant is reported as rare in other European populations. We therefore screened a control sample of healthy women and found lower frequency compared to the patients (16.6%).

Another frequently observed polymorphic variant rs799917 (L871P) at position 1941 of BRCA1 exon 11 was more often found in healthy controls (38,5%), compared to breast cancer patients (21.05%), but the difference did not reach statistical significance.

Conclusions: Our preliminary studies did not identify causative mutations in exon 11 of BRCA1. The frequent Q356R polymorphism in the Bulgarian population may have some role for breast cancer susceptibility, although there is just a trend in the association study. This is in agreement with a previous finding that the simultaneous presence of this rare mutation and other missense mutation S1512I may be associated with the breast cancer phenotype in 2 Cypriot families. An extended case-control study of Bulgarian breast cancer families and controls is necessary to confirm this hypothesis.

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Thymidylate synthase genotypes as a prognostic factor in non-small cell lung cancer patients

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The thymidylate synthase (TS) enzyme plays a key role in de novo DNA synthesis, essential for DNA replication and repair, thus it is important in cell proliferation and it is a target for several chemotherapy agents. The thymidylate synthase (TYMS) gene, mapped on chromosome 18, is highly polymorphic in Humans. Three polymorphisms in the TYMS untranslated regions (UTRs) have been identified. TYMS enhancer region (TSER) polymorphism is a 28bp tandem repeat that can influence TYMS transcription or translation. A novel functional G>C single nucleotide polymorphism (SNP) present in the second repeat of 3R alleles has also been identified and the translation efficiency of the 3RC allele is equivalent to 2R allele. TYMS 1494del6, a 6bp deletion at nucleotide 1494 in the 3'UTR has been associated with decreased mRNA stability and lower intratumoral TS expression.

The main goal of this study was to analyse the influence of functional TYMS polymorphisms as a prognostic marker in a series of non-small cell lung cancer (NSCLC) patients receiving, as first line chemotherapy regimens, an association of a platinum with a no-platinum agent.

DNA was extracted from peripheral blood leukocytes of 152 NSCLC patients from the North region of Portugal, admitted at the Portuguese Institute of Oncology. TYMS genotypes were detected by PCR-RFLP techniques. Analysis of TYMS TSER and 3RG>C SNP polymorphisms were stratified according to the functional status in low expression (2R/2R, 2R/3RC and 3RC/3RC) and high expression (2R/3RG, 3RG/3RC and 3RG/3RG) genetic profile. TYMS 1494del6 genotypes were analysed according to the recessive model (homozygous ins versus del carriers). Survival was compared between different genotypes at 12 months, 36 months and for overall survival, using multivariate Cox proportional hazards regression models. Multivariate Cox models were adjusted for NSCLC tumour stage and histological type. Hazard ratio (HR) and 95% Confidence Interval (95%CI) were calculated.

Our results showed that in TSER polymorphisms the high expression group was associated with a better outcome at 12 months (HR=0.12; 95%CI, 0.02-0.91; P=0.040) and at 36 months (HR=0.47; 95%CI, 0.21-1.05; P=0.066). TYMS 1494del6 polymorphism was associated with a better outcome at 12 months (HR=0.20; 95%CI, 0.07-0.60; P=0.004, for the recessive model).

Analysis of TYMS polymorphisms may be useful as a prognostic factor in NSCLC patients. Nevertheless, since the genetic variants associated with better outcome have controversial end points related with TS expression levels, our results further suggest that the clinical usefulness of TYMS genotyping should be correlated with protein assessment and merits caution.

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Functional genetic polymorphisms in the leptin pathway are associated with progression free interval and overall survival in ovarian cancer patients

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Background: Leptin is a pleiotrophic hormone with proliferative and angiogenic potential. Its receptor (LEPR) was found in epithelial ovarian cells and the leptin/LEPR pathway is known to stimulate ovarian cancer (OvCa) cells growth. The G-to-A substitution at locus -2548 in leptin gene (LEP) promoter region has been found to directly affect the LEP transcription rate in vitro and leptin levels in vivo, while a Gln-to-Arg substitution at codon 223 in LEPR gene results in higher leptin binding affinity. Therefore, we sought to determine the association of LEP and LEPR polymorphisms with OvCa.

Materials and Methods: We genotyped the LEP -2548 G>A and the LEPR Gln223Arg polymorphisms in histologically confirmed ovarian cancer patients (n=189) (54.4±12.8 years of age) by PCR-RFLP. Genotypes of these polymorphisms were combined into 3 categories according to the functional leptin/LEPR signaling phenotype: low, intermediate and high signalling capacity genetic profile (according to gene expression levels and binding affinity). Multivariate Cox regression model included as covariates the disease histological type, grade, residual tumor after surgery, stage, age, menopausal status and lymphatic invasion.

Results: Univariate analysis using Kaplan Meier function plots and Breslow test evidenced a significant cumulative probability for earlier disease recurrence in LEPR Arg homozygous carriers (P=0.031).

Cumulatively, multivariate Cox regression showed an increased hazard for disease relapse in Arg/Arg carriers (HR=4.19, CI=1.44-12.18, P=0.008). In LEP/LEPR combination genetic profile analysis we found that intermediate/high signalling carriers had higher risk for OvCa relapse (HR=2.49, CI=1.04-5.98, P=0.041), compared with low signalling genetic profile carriers.

Overall survival univariate analysis revealed a shorter survival in Arg homozygous and in intermediate/high leptin/LEPR signalling genetic profile carriers (P=0.001 and P=0.004, respectively). Multivariate analysis showed a worst outcome for LEPR Arg/Arg and intermediate/high leptin/LEPR signalling genetic profile carriers (HR=3.54, CI=1.45-8.64, P=0.005 and HR=3.44, CI=1.52-7.77, P=0.003, respectively).

Conclusions: Functional polymorphisms of the leptin pathway, alone or in combination, may impact as predictive and prognostic molecular markers in OvCa. A role for the leptin/LEPR pathway in OvCa is proposed by observations from the present study.

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Identification of tp53 gene mutations in sporadic, familial breast cancer cases and Li.Fraumeni syndrome. Is codon 72 polymorphism of tp53 gene can be consider as an important mutation marker

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Objective: What is the mutation spectrum of tp53 gene in Pakistani breast cancer patients and Li.Fraumeni syndrom. It is the first study of its type from Pakistan.

Materials and Methods: 100 sporadic breast cancer patients, 3 families of breast cancer background and one Li.Fraumeni syndrome family was taken as subject. Out of sporadic cancer patients, from 50 patients only blood was taken as a sample and from remaining 50 patients, three type of specimens i.e. blood, normal tissue and tumor tissue has been collected from each patient. DNA was isolated from each specimen.

We analyzed 5-8 coding exons of the tp53 gene by Temporal Temperature Gradient Gel Electrophoresis (TTGE) and direct sequencing of Polymerase Chain Reaction (PCR) product. DNA from blood of members of 3 families and a Li.Fraumeni syndrome family was analyzed for genetic variations in codon 72 in exon 4 of tp53 gene using Restriction Fragment Length Polymorphism

Results: Out of these 100 sporadic cancer patients, one patient's tumor tissue has showed mutation (Pro278Ser) in exon 8. This mutated person's normal tissue and blood showed no mutation and confirmed the importance of taking three type of specimens from each patient for comparison. Out of 3 families of breast cancer background, tp53 gene mutation was not detected. In case of Li.Fraumeni syndrome (LFS), one member of family no. 1(Li.F1), with a strong history of LFS in two generations, shows mutation in exon 8 of tp53 gene. In case of Li.F1 sample we identified 3 mutations in same exon (exon 8). The three mutations include (Asn268His),(Glu286Asp) and (deletion292).

Codon 72 variation Pro/Pro and Arg/Pro was about 50% each. There are codon 72 variations in Li.Fraumeni syndrome family (Li.F1) which is already proved positive for p53 gene mutations.

Conclusions: tp53 mutations shows more dominant results in case sporadic breast cancer and Li.Fraumeni syndrome. Total number of patients for mutation detection is also an important factor and we are enhancing our data.. Codon 72 variation of tp53 gene is proved as an important mutation marker. Relationship of Li. Fraumeni syndrome to tp53 gene mutations could be analyzed in scenario of codon 72 polymorphism.

Significance of work: Our study emphasizes the importance of mutational analyses of the tp53 gene, particularly in young patients with malignancies.

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Serum EGFR and serum HER2 in patients with triple negative breast cancer

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PURPOSE: Triple negative breast cancer (ER-PR-HER2 negative) is not very common expecially in Europe. This special type of breast cancer has very poor prognosis and his therapeutic approach has become a major problem. From clinical studies this type overexpress EGFR in tissue and so maybe anti-EGFR treatments can be very useful in these patients. In our study we tried to measure serum EGFR and serum HER2 in an effort to find prognostic factors for this special subgroup.

Patients and methods: During the last five years we have operated 76 patients with triple negative breast cancer (ER-PR-C-erb-B2 negative) and two patiens had core biopsy and preoperative chemotherapy. The mean age of the patients was 59,41±10.5. The tumor size was <2 cm in 30 patients, >2 cm and <5 cm in 19 patients, >5cm in 27 patients. 17 patients had multifocal breast cancer. 29 patients were node negative, 14 had <3 positive lymph node and 33 had >3 positive lymph node. The histological type was invasive ductal carcinoma in 67 patients, 5 medullary carcinoma, 3 mucinous, 2 invasive lobular carcinoma and 1 patient had DCIS. All the patients had chemotherapy and radiotherapy after the surgical treatment. 14 patients had distant metastases, 6 during the first year of their follow-up and 8 during the second year and 5 patients died during the first two years of their follow-up. From the 73 patients who they are alive 29 patients are in follow-up for less than one year, 10 patients for >2 years, 12 patients for >3 years and 22 patients for > 4years. The percentage of early distant metastases and death in our study is 17,5% and 6,4%. Serum EGFR and serum HER2 were measured in 73 patients who were alive with the method of ELISA.

RESULTS: From our results serum EGFR was not overexpressed in patients with triple negative breast cancer (mean value 0.071±0,0,021). Although it was expressed in all patients. HER2 was overexpressed in 8 patients with tripple negative breast cancer.

CONCLUSION: Although triple negative breast cancer overexpress EGFR in tissue, in serum EGFR is not overexpressed. On the other side we need more patients to estimate the fact that some patients overexpress HER2 in serum although they are HER2 negative in tissue. The study is continued with more patients and more measurements of the two factors during their follow-up.

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Increased intake of fruits and vegetables high in vitamin C and fibre is associated with decreased risk of renal cell carcinoma in the US

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Although renal cell carcinoma (RCC) accounts for only 3% of adult malignancies in the USA its incidence has been increasing in the U.S. for the last thirty years, from 12 to 18 per 100,000 among white men and from 5 to 18 per 100,000 among white women. The increase cannot be fully explained by early detection of pre-symptomatic tumours. The reported ongoing epidemic of obesity in the USA and/or the increase in hypertension and diabetes may explain part of this increase, which occurred despite a drop in smoking rates. Although obesity, hypertension, and diabetes have consistently been associated with RCC risk, few studies have tried to disentangle the effects of obesity from changed dietary intake and lack of physical activity.

A population-based case-control study of 406 cases and 2,434 controls aged 40 to 85 years was conducted in Iowa in 1986-89. For 323 cases and 1820 controls from this study, information on dietary intake and other lifestyle factors was obtained using a mailed questionnaire. Cancer risks were estimated by odds ratios (OR) and 95% confidence intervals (CI), taking into account other known risk factors, especially obesity, dietary fat, hypertension, alcohol intake, physical activity and smoking habits.

High intake of vitamin C measured either by food group; raw fruit and vegetables (OR=0.4; CI=0.3-0.7, ptrend <0.001) or by nutrient: vitamin C (OR=0.4; CI=0.2-0.6) ptrend <0.001), was associated with a lower risk of RCC, when the highest quartile of intake was compared to the bottom quartile. When the nutrient fibre was differentiated by source, fruit and vegetable fibre intake showed similar significant trends but not grain fibre. Analysis of flavenoids is underway. Similar associations were found with high intake of folate, xanthin, cryptoxanthin and lycopene but not with α and β-carotene, lutein and vitamin E. However, when stratified by gender, RCC rates were only associated with low intakes of vitamin C, xanthin, cryptoxanthin, vitamin E and lutein in women.

As increased fruit and vegetable intake is an important public health message for other cancers and coronary heart disease prevention, these findings have implications for dietary, clinical and public health interventions.