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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).