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Polymorphisms in cellular transporters and their relevance to cancer risk and cancer therapy

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Two members of the ATP binding cassette (ABC) transporter family, ABCC3 (multidrug resistance-associated protein 3, MRP3) and ABCG2 (breast cancer resistance protein, BCRP), contribute to the cellular efflux of xenobiotics. As their substrates include various carcinogens and drugs used in chemotherapy, variations of these transporters could influence both cancer risk as well as outcome of chemotherapy. The concentrative nucleoside transporter 1 (CNT1) is involved in the uptake of pyrimidine nucleosides, which affects the distribution of nucleoside analogs used in chemotherapy, such as gemcitabine or cytarabine.

In two different studies the impact of single nucleotide polymorphisms (SNPs) in *ABCC3*, *ABCG2* and *CNT1* was investigated.

As part of a hospital-based case-control study, DNA isolated from blood samples of 1078 Caucasian patients with primary lung cancer and 588 controls without malignancy was genotyped. For a subgroup of 349 Caucasian patients (161 small cell lung cancer (SCLC), 187 non-small cell lung cancer (NSCLC) and 1 mixed) receiving first-line chemotherapy 3 different endpoints were analysed: response after the 2nd cycle, progression free survival (PFS), and overall survival (OS). A second study included patients with primary acute myeloid leukaemia (AML)
(68 Jewish and 44 Arab) and healthy controls (111 Jewish, 70 Arab), all recruited at the Hadassah University Hospital, Jerusalem. The impact of SNPs in transporter genes on AML risk as well as on remission and survival of AML patients treated with chemotherapy was analysed.

Genotyping was performed using fluorescence-based melting curve analysis (LightCycler). The risk of developing the respective cancer was analysed using multiple logistic regression, calculating odds ratios (OR) by comparing genotype frequencies in cancer patients and controls. Likewise the prognostic value of the SNPs was calculated comparing genotype frequencies in responders and non-responders. In addition; hazard ratio estimates (HR) for PFS and for OS were calculated and Kaplan-Meier curves were plotted.

No significant influence of SNPs on the risk to develop one of the two cancer types investigated was found.

In the group of all lung cancer patients receiving chemotherapy, none of the investigated polymorphisms modified response statistically significantly. SCLC patients carrying the *ABCC3 -211T* allele show a significantly worse PFS (HR: 1.79; 95% confidence interval (CI) 1.13-2.82). In a subgroup analysis the carriers of the *ABCG2 421A*-allele who were treated with platinum-based drugs showed a significantly worse OS (HR: 1.60; 95% CI 1.04-2.47;

n=256). AML patients with the homozygotes *CC*-genotype regarding *ABCC3 C-211T* are less favoured in reaching remission (p=0.020) and in overall survival (p=0.044), the other SNPs investigated had no significant effect.

In both studies the SNP *ABCC3 C-211T* was identified as a relevant factor for treatment outcome, however, the preferable allele varies between the two studies. Further studies are needed to elucidate the reasons for the divergence, which could be due to differences in the cancer site, in the treatment protocol, or in the ethnicity of the patients.

A potential phenotypic effect of SNPs, which is less apparent from the genomic data alone, manifests itself in a variation of the RNA splicing pattern. Splice-relevant SNPs are attractive for future molecular epidemiological studies as the phenotypic effect can be expected to be high. Using the computer program AASsites we performed *in-silico* analysis to identify such SNPs. AASsites was created using an analysis pipeline design. It calls two other programs, Geneid and Genscan, which both predict the intron-exon structure of a gene's mRNA based on the DNA sequence data. Predictions based on the wild-type sequence and on the polymorphic sequence are generated and then compared, thereby identifying potential candidates for splicing-relevant SNPs.

35 genes, mostly relevant to xenobiotic metabolism or transport, were selected for screening. Then within the sequences of these genes 6994 polymorphisms described in the NCBI SNP database were evaluated using the computer program AASsites. As a result 44 SNPs with a potential impact on splicing were identified. One of these SNPs, *CYP21A2 A/C656G* (rs6467), had previously been described in the literature as leading to an intron 2 splice variation. 6 SNPs are not present in Caucasians and 3 have a frequency of >0.05 making them unsuitable for further analysis. 3 SNPs with an adequate frequency were rejected as putative candidate polymorphisms for other reasons. For the remaining 31 SNPs there is no information about the allelic frequency in Caucasians. *In vitro* validation of some of the most promising potential splice variants is still required to verify the applicability of these *in silico* results.