

10053- Association between Head and Neck Cancer and Microsomal Epoxide Hydrolase Genotypes
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Squamous cell cancer of the head and neck (SCCHN) is a group of epithelial cancers of the upper aerodigestive tract which are mainly caused by smoking and other lifestyle factors. Polycyclic aromatic hydrocarbons (PAH), a tobacco smoke constituent, are metabolized in a cytochrome P450-mediated process to highly reactive epoxides which are converted by human microsomal epoxide hydrolase (mEH) to trans-dihydrodiols. Sequence variations in the gene EPHX1 encoding for mEH may alter the enzyme activity and thus modulate the risk of PAH for tobacco-related cancer, especially in smokers. mEH has polymorphic variants with either a Tyr (Y) or His (H) substitution in codon 113 and either a His (H) or Arg (R) substitution in codon 139. EPHX1 genotype combinations, according to in-vitro expression studies of cDNA published in 1994, were used in literature to predict a putative low, medium and high mEH activity. We conducted a case-control study with 280 SCCHN cases and 289 non-cancer controls to estimate the SCCHN risk of the Y113H and H139R EPHX1 polymorphisms with respect to smoking habits and putative enzyme activity. Genomic DNA isolated from whole blood was genotyped with a LightCycler™ instrument (Roche, Mannheim, Germany). We found allele frequencies of 31% for the 113H allele and of 21% for the 139R allele in controls which correspond to literature. We could not detect overall SCCHN risks of the genotypes (for 113YH OR 0.83, 95% CI 0.56-1.23; for 113HH OR 0.89, 95% CI 0.45-1.75, for 139HR OR 0.75, 95% CI 0.51-1.12, for 139RR OR 1.38, 95% 0.50-3.80), but a lower risk of the 139HR genotype in smokers (OR 0.57; 95% CI 0.34-0.95). There was no SCCHN excess risk for genotype combinations according to a putative medium or high enzyme activity, but heterogeneity within these categories among smokers using the 113HH/139HH genotype combination, assigned to the putative low enzyme activity category, as reference (Wald χ^2 test P=0.02). With the exception of 113YY/139RR, the other genotype combinations were associated with a lower risk than 113HH/139HH. Especially the putative high genotype combinations 113YY/139HR and 113YH/139RR were associated with a significantly lower risk (OR 0.30; 95% CI 0.09-0.97 and OR 0.10; 95% CI 0.01-0.93 respectively). The two variant amino acids are far away from the catalytic center of the protein. Meanwhile, new sequence variations and data on mEH expression and enzyme activity have been reported. We conclude that the impact of the EPHX1 sequence variations on the enzyme activity is still not yet conclusive for genotype-phenotype relations.

10194- EFFECTS OF DNA REPAIR AND METABOLIC GENE POLYMORPHISMS ON
MUTANT ONCOPROTEINS IN WORKERS EXPOSED TO VINYL CHLORIDE
MONOMER

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The presence of mutant Asp13-K-ras protein, mutant p53 protein and anti-p53 antibody have been reported to be associated with vinyl chloride monomer (VCM)-related cancers. To investigate the role of polymorphisms of metabolic and DNA repair genes on VCM-induced oncoproteins, we examined the plasma samples of 218 male workers occupationally exposed to VCM. Plasma mutant p53 protein and anti-p53 antibody were detected with enzyme-linked immunosorbent assay (ELISA), and Asp13-K-ras proteins were detected using enhanced chemiluminescence Western blotting. Genotypes of cytochrome P450 2E1 (CYP2E1), aldehyde dehydrogenase 2 (ALDH2), glutathione S-transferase T1 (GSTT1) and X-ray repair cross-complementing group 1 (XRCC1, exon 10) were identified using the polymerase chain reaction (PCR). The results revealed that the plasma mutant p53 protein was positive in 10.1% of workers, anti-p53 antibody was positive in 5% of workers and Asp13-K-ras protein was positive in 10.1% of workers. High VCM exposure group (≥ 40 ppm-years) had significantly higher mutant oncoprotein (mutant p53 protein, anti-p53 antibody or Asp-13-K-ras protein) expression as compared to low VCM exposure group (< 40 ppm-years) (OR=2.0, 95%CI=1.0-3.8). Amongst high exposure workers, subjects with XRCC1 Gln/Gln genotypes demonstrated significantly higher risk of oncoprotein expression as compared to those with XRCC1 Arg/Arg or Arg/Gln variants (OR=8.5, 95%CI=1.9-38.9) after adjusting for potential confounders. Moreover, there was an interaction between VCM exposure and XRCC1 polymorphisms on oncoprotein expression ($p=0.06$). However, there was no interaction between VCM exposure and genotypes of CYP2E1, GSTM1, and ALDH2 on oncoproteins. Our results suggest that susceptible XRCC1 genotype may modulate the mutation of p53 and K-ras gene amongst VCM-exposed workers.

10203- Tobacco smoke exposure, cytochrome P450 1A1 polymorphisms, and breast cancer risk
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Environmental exposure to carcinogens may contribute to increasing breast cancer rates and one class of chemicals that has received much attention are the polycyclic aromatic hydrocarbons (PAH) that are ubiquitous in the environment and occur in cigarette smoke. The cytochrome P450 1A1 (CYP1A1) gene which codes for an enzyme with aryl hydrocarbon hydroxylase activity is involved in metabolism of PAHs. Genotypic variants of CYP1A1 have been associated with altered aryl hydrocarbon hydroxylase activity which may modify the risk for breast cancer associated with tobacco smoke exposure. Although recent studies suggest that passive smokers should be separated from the non-exposed group, the effect of CYP1A1 gene variants on the association of active and passive smoking with breast cancer has not been studied. In a population-based case-control study of breast cancer by age 50 in Germany, we determined the *2A, *2B, and *4 polymorphisms in CYP1A1 among 421 incident breast cancer cases and 888 matched controls using fluorescence based capillary PCR followed by melting curve analysis (LightCycler). Odds ratios (ORs) and 95% confidence intervals (CIs) were used to quantify the risk of breast cancer among subjects who had at least one variant allele relative to subjects who were homozygous for the wild-type allele, using conditional logistic regression. No overall increase in breast cancer risk with the variant CYP1A1 genotypes was apparent (OR(CYP1A1*2A): 0.9, 95% CI 0.6-1.4; OR(CYP1A1*2B): 1.0, 95% CI 0.6-1.7; and OR(CYP1A1*4): 1.0, 95% CI 0.6-1.5). Compared to women never regularly exposed to tobacco smoke, multivariate OR for active cigarette smoking were not significantly different among women homozygous for the wild-type allele (1.4, CI 0.9-2.2) and in those with at least one variant allele (0.9, CI 0.4-2.2). ORs were higher for 11+ than for 1-10 pack-years of cigarettes smoked only among women homozygous for the wild-type allele (interaction $p=0.3$). Among nonsmokers, passive smoking was associated with a significantly increased risk for breast cancer among women homozygous for the wild-type allele (1.7, CI: 1.0-2.8) but not for carriers of at least one variant allele (1.3, CI: 0.5-3.4) (interaction $p=0.2$). Compared to non-users, ORs for ever oral contraceptive use were 1.4 (CI 0.7-2.8) in variant allele carriers and 1.0 (CI 0.7-1.4) in homozygous carriers of the wild-type allele (interaction $p=0.3$). These data from predominantly premenopausal women suggest that genotypic variants of CYP1A1 alone do not strongly modify the association between tobacco smoke exposure and breast cancer, but possible interactions of CYP1A1 with estrogen metabolism may play a role and therefore require further study.

10215- Association of hepatitis virus infection, alcohol consumption and serum vitamin A level with urinary 8-hydroxydeoxyguanosine in chemical workers

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Urinary 8-hydroxydeoxyguanosine (8-OHdG) DNA adduct has been used as a biomarker in epidemiological studies. However, the determinants for urinary 8-OHdG are not clear. We tested urinary 8-OHdG levels in 205 male workers who had been exposed to vinyl chloride monomer. Epidemiological information was obtained by an interviewer-administered questionnaire. Hepatitis B surface antigen (HBsAg) and anti-Hepatitis C antibody (anti-HCV) were also determined by immunoassay and plasma antioxidants including vitamin A, α - and β -carotenes and vitamin E were assayed by HPLC. Median of urinary 8-OHdG was 9.8 ng/mg creatinine (range, 1.4-60.1). Those who consumed alcohol had higher urinary 8-OHdG than those who did not ($P < 0.01$). However, there was no dose-response between amount of alcohol consumption and urinary 8-OHdG. Workers with positive HBsAg had higher urinary 8-OHdG than those without. The difference of urinary 8-OHdG was even more prominent between those with anti-HCV and those without. Plasma vitamin A level was also found to be positively associated with urinary 8-OHdG, whereas plasma α - and β -carotenes and vitamin E levels were not associated with urinary 8-OHdG. However, we did not observe an association of urinary 8-OHdG with age, smoking, BMI or VCM exposure. The results suggest that active inflammation of hepatitis B and C, alcohol consumption and vitamin A can induce oxidative stress. The lack of association between urinary 8-OHdG and potential determinants was probably due to a wide variation in individual antioxidant enzyme system. Thus, we conclude that potential determinants need to be considered in the epidemiological studies when urinary 8-OHdG is used as a biomarker.

10223- EFFECTS OF DNA REPAIR AND METABOLIC GENE POLYMORPHISMS ON
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The presence of mutant Asp13-K-ras protein, mutant p53 protein and anti-p53 antibody have been reported to be associated with vinyl chloride monomer (VCM)-related cancers. To investigate the role of polymorphisms of metabolic and DNA repair genes on VCM-induced oncoproteins, we examined the plasma samples of 218 male workers occupationally exposed to VCM. Plasma mutant p53 protein and anti-p53 antibody were detected with enzyme-linked immunosorbent assay (ELISA), and Asp13-K-ras proteins were detected using enhanced chemiluminescence Western blotting. Genotypes of cytochrome P450 2E1 (CYP2E1), aldehyde dehydrogenase 2 (ALDH2), glutathione S-transferase T1 (GSTT1) and X-ray repair cross-complementing group 1 (XRCC1, exon 10) were identified using the polymerase chain reaction (PCR). The results revealed that the plasma mutant p53 protein was positive in 10.1% of workers, anti-p53 antibody was positive in 5% of workers and Asp13-K-ras protein was positive in 10.1% of workers. High VCM exposure group (≥ 40 ppm-years) had significantly higher mutant oncoprotein (mutant p53 protein, anti-p53 antibody or Asp-13-K-ras protein) expression as compared to low VCM exposure group (< 40 ppm-years) (OR=2.0, 95%CI=1.0-3.8). Amongst high exposure workers, subjects with XRCC1 Gln/Gln genotypes demonstrated significantly higher risk of oncoprotein expression as compared to those with XRCC1 Arg/Arg or Arg/Gln variants (OR=8.5, 95%CI=1.9-38.9) after adjusting for potential confounders. Moreover, there was an interaction between VCM exposure and XRCC1 polymorphisms on oncoprotein expression ($p=0.06$). However, there was no interaction between VCM exposure and genotypes of CYP2E1, GSTM1, and ALDH2 on oncoproteins. Our results suggest that susceptible XRCC1 genotype may modulate the mutation of p53 and K-ras gene amongst VCM-exposed workers.

10272- Exposure to indoor air pollutants and glutathion *S*-transferase polymorphisms in a Hungarian asthmatic children population

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Indoor air quality and potential individual susceptibility markers were investigated in a pilot study to reveal relationships between indoor air pollution and respiratory health of pupils aged 9-11 years. Indoor concentrations of gaseous air pollutants were measured by passive monitors in the children's home. Glutathione *S*-transferase gene polymorphisms (*GSTM1*, *GSTP1*) were determined by PCR-based methods from blood samples from 128 children with allergy/asthma or obstructive bronchitis, and 94 pupils without allergic symptoms and respiratory problems. Independent t-tests and chi-square test were used for statistical analyses. The *GSTM1* and *GSTP1* genotype frequencies were similar in our total study population to those observed in a healthy adult Hungarian population. We did not find significant differences in allelic frequencies of *GSTM1* and *GSTP1* genotypes between cases and controls. When stratification of the cases and controls occurred according to high and low indoor NO₂ and benzene exposures, (thresholds: 85 µg/m³ for NO₂, and 40 µg/m³ for benzene), respectively, we observed a shift towards higher percentage of individuals with *GSTM1* positive genotype among the cases compared to the corresponding controls in the high NO₂ .group (p=0.061). Data showed significant differences in *GSTP1* genotype frequencies between asthmatic/allergic pupils with lower benzene exposure compared to the corresponding healthy subgroup (chi²=6.55, p=0.037). Glutathione *S*-transferases may have special roles in inflammatory mechanisms against airway pollutants, however, further research is needed to clarify their suggested role in susceptibility to childhood respiratory diseases.

10645- The skin is under a high rate of proliferation and an ideal model to investigate the interaction of exogenous exposures and genetic variation in repair efficiency on the development of non-melanoma skin cancer (NMSC). ERCC2 variations at g.22541 A>C (Arg156Arg) and g.35931 A>C (Lys751Gln) could alter the repair efficiency and thus constitute a risk factor. We conducted a population-based case-control study with 264 cases and 286 controls to investigate joint effects of genetic susceptibility with exposure to environmental factors such as arsenic and UV exposure on NMSC development. gDNA of the study subjects was extracted from buccal swaps. Mutation detection was performed with LightCyclerTM technology. A binary variable 'sensitive skin' was constructed with respect to frequent sunburns, many freckles, or light color of eyes and hair. Odds ratios (OR) with 95% confidence intervals (CI) were calculated with logistic regression analysis, conditional on age and gender. Skin sensitivity was a significant NMSC risk factor (OR 1.8, 95% CI 1.2-2.7), also hair color and other constitutional factors. High exposure to arsenic in soil, measured in a random 50% subsample, was marginally significant using the median as cutoff (OR 1.5, 95% CI 1.0-2.4). There were no significant main effects of the ERCC2 polymorphisms, but 139 persons were heterozygous in both genotypes (99 expected) and no person had the combination 751GlnGln/22541CC (20 expected), indicating either selection pressure or a non-random distribution of missing values. A stratification by the environmental factor did not reveal significant genetic effects. Methodological problems arise from the low joint prevalence of the genetic and environmental factor and from random variation if stratification overstresses the sample size.

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10691- Chromosomal aberrations as biomarker of early effects.

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Introduction: The process of validation of biomarker of early effects requires several steps. From the epidemiologic point of view a proof of statistical association between exposure and quantity of biomarker and association between biomarker and ultimate effects are crucial requirements. In case of frequency of chromosomal aberrations (CAs) determined in peripheral blood lymphocytes (PBL) the association with different types of carcinogenic exposures became evident during 70's and 80's and cytogenetic assay has been established as a valuable tool for monitoring of exposures to many industrial and latter also environmental clastogens. However, the frequency of CAs in surrogate tissues, most often in PBL, has been conceptualized as a biomarker of early effects rather than biomarker of exposure and an empirical evidence on the association between frequency of CAs in PBL and incidence of neoplasm was missing until the observations of the positive association in 90's (Brooger et al 1990, Bonassi et al 1995, Liou et al 1999). Also in our previous research we observed the positive association between cancer incidence and CAs (Smerhovsky et al. 2001, 2002). However, our finding was limited on a group of radon-exposed miners and we have failed to demonstrate similar association in workers exposed to chemical carcinogens.

Methods: Now we report results of the enlarged retrospective follow-up study, which includes 11,986 participants who underwent altogether 20,783 cytogenetic assays mostly because of occupational exposures to different chemical mutagens and carcinogens. The total follow-up time was 115,168 person-years. During the follow-up period we identified 445 cases of cancer and carcinomas in situ in the cohort. The mean frequency of aberrant cells was 2.0%.

Results: To model the association between CAs and cancer incidence we used Cox's regression. The most interesting finding seems to be the specificity of the association of interest with respect to diagnostic groups as well as type of aberrations. The occurrence of chromatid exchanges in participants increased risk of lung cancer (RR=2.40, 95%CI 1.37-4.22, P<0.00). Participants with high frequency (3rd tercil) of chromosome breaks were in higher risk of cancer of lymphoid and haematopoietic tissues (RR = 3.81, 95%CI 1.40 - 10.37, P<0.01). Finally, 1% increase in percentage of aberrant cells was followed by 58% increase in incidence of digestive organs cancer. All models accounted for the age of participants at first assay.

Conclusions: The presented study is the first attempt to analyze the effect of increased frequency of different types of CAs on subsequent risk of cancer in occupationally exposed population using the cohort design. The results of the study bring another piece of evidence on the validity of CAs determined in PBL as a biomarker of early effects of clastogenic exposures.

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10717- The effect of environmental exposure to airborne particulate-bound polycyclic aromatic hydrocarbons (PAHs) on DNA adduct levels.

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Epidemiologic studies indicate that prolonged exposure to high air pollution levels is associated with increased risk of cancer, especially of lung cancer. The capital city of Prague becomes one of the most polluted localities of the Czech Republic because of geographic surroundings, inner-city architecture and high traffic. Therefore, the effect of exposure to PAHs adsorbed to respirable air particles (<2.5 µm) on DNA adduct levels was studied on the group of policemen (males, aged 22-50 years) working in the middle of the City and spending >8h outdoors (EXP, N=53). The matched healthy volunteers spending >90% daily time indoors were chosen as Controls (CON, N=52). Ambient air particles (PM10, PM2.5) and carcinogenic PAHs (carc. PAHs) were daily monitored using VAPS samplers, personal exposure was evaluated using personal samplers during working shift prior collection of biological samples. DNA adducts were analysed in lymphocytes by ³²P-postlabeling assay, cotinine in urine to control for exposure to tobacco smoke by radioimmunoassay, plasma levels of vitamins A, E and C by HPLC, cholesterol and triglycerides using commercial kits. During the sampling period (February 2000) ambient air pollution was as follows: PM10 32-55 µg/m³, PM2.5 27-38 µg/m³, carc. PAHs 18-22 ng/m³; personal exposure to carc. PAHs: 12.0±11.1 ng/m³ and 6.2±3.5 ng/m³, for EXP and CON groups, respectively. The DNA adduct levels did not significantly differ between EXP and CON groups (0.92±0.28 vs. 0.82±0.23 adducts/10⁸ nucleotides). The significant difference was observed between smokers and nonsmokers within both groups (EXP: 1.04±0.28 vs. 0.86±0.26 P=0.022; CON: 1.14±0.15 vs. 0.76±0.20, P<0.001). Based on the individual values, the significant positive association was found between DNA adduct and cotinine levels (r=0.368, P<0.001) and negative association between DNA adduct and vitamin C levels (r=-0.290, P=0.004). No relationship between short-term exposure to PAHs as evaluated by personal monitors during working shift and DNA adduct levels was observed. The results suggest that prolonged exposure to tobacco smoke possess more significant effect on steady-state levels of DNA adducts in lymphocytes than exposure to urban airborne particulate-bound PAHs.

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10720- FISH analysis of chromosome breakage as biomarker of genotoxicity of urban air pollution.

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As a result of geographic surroundings and a high traffic it is believed that Prague became one of the most polluted region of the Czech Republic. Therefore, the effect of air pollution was studied on the groups of policemen working in the middle of the City and spending > 8 h outdoors (EXP, N=53). The matched healthy volunteers, spending > 90% daily time indoors, were chosen as Controls (CON, N=52). Ambient air particles (PM10, PM2.5) and carcinogenic polycyclic aromatic hydrocarbons (carc. PAHs) were monitored using VAPS sampler, personal exposure was evaluated using personal samplers during working shift. The effect of PAHs exposure was studied by conventional cytogenetic analysis and fluorescent in situ hybridization (FISH). During the sampling period ambient air pollution was as follows: PM10 32-55 $\mu\text{g}/\text{m}^3$, PM2.5 27-38 $\mu\text{g}/\text{m}^3$, carc. PAHs 18-22 ng/m^3 ; personal exposure to carc. PAHs: 12 ng/m^3 and 6.2 ng/m^3 , for EXP and CON groups, respectively. The frequency of chromosomal aberrations by conventional method (% AB.C.) did not differ between EXP and CON groups (2.31% vs. 1.98 % AB.C). However, these values are 2-fold higher than spontaneous level of chromosomal aberrations in the Czech Republic. Using FISH technique and probes for chromosomes 1 and 4 (Cambio, UK) the genomic frequency of translocations calculated as $F_G/100$ was 1.74 and 1.35 for EXP and CON, respectively. The significant difference in $F_G/100$ was observed between smokers (S) and nonsmokers (NS) in CON (2.51 vs. 1.16, $P<0.005$). Analyzing the breakage events by FISH (B/1000) the difference between EXP and CON groups was significant (5.6 vs. 4.2, $P<0.05$, EXP vs. CON), as well as between S and NS in both groups (EXP: 7.1 vs. 4.8, $P<0.05$; CON: 7.7 vs. 3.6, $P<0.005$). Our results using FISH method indicate that contemporary exposure to 18-22 ng carc. PAHs/ m^3 may increase the breakage event frequency in chromosomes of policemen working all day shift outdoors. FISH painting method seems to be more sensitive method than conventional analysis of chromosomal aberrations to evaluate the clastogenic activity of urban air pollution.

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