ASSOCIATION BETWEEN GENETIC POLYMORPHISMS IN PAH-METABOLIZING ENZYMES, AND DATA FROM THE BIOMONITORING AND AMBIENT AIR MONITORING OF PAH-EXPOSED WORKERS

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The activation and conjugation of polycyclic aromatic hydrocarbons (PAH) are regulated by polymorphic genes. We investigated single nucleotide polymorphisms (SNPs) in the genes *CYP1A1*, *CYP1B1*, *CYP3A4*, *mEH*, *GSTM1*, *GSTT1* and *GSTP1* using real-time PCR techniques and their influence on biological markers (1-hydroxypyrene (1-OHP), hydroxy-phenanthrenes (OHPH)) in relation to 8 hour working shift personal ambient monitoring of 16 different PAH. Biomonitoring markers were analysed by HPLC in post-shift urine samples of 109 occupationally PAH-exposed male workers (production of refractories: n=60, graphite-electrode production: n=28, coke oven: n=13, workers handling coal tar: n=8). The mean age was 37 years (range: 19-62 years). Smoking habits were assessed by both questionnaire and urinary cotinine. 72% of the workers were smokers. The PAH concentrations of total particulate PAH, air-borne pyrene and phenanthrene concentrations ranged between 0.97 and 1,848 µg/m^3 (median: 23.5 µg/m^3), 0.048 and 300.6 µg/m^3 (median: 1.48 µg/m^3) and 0.07 and 176.02 µg/m^3 (median: 4.24 µg/m^3) respectively. Concentrations of biomarkers ranged between 0.06 and 117.25 µmol/mol creatinine (1-OHP) as well as 0.69 and 71.11 µmol/mol creatinine (OHPH). The ratio of the sum of urinary OHPH and the phenanthrene exposure, was significantly associated with the genetic variation g.3801T>C (**2A*) in *CYP1A1* (p=0.03; one-sided exact Wilcoxon rank-sum test). All PAH-exposed workers (N=109, one-sided

p=0.05), and the subgroup of PAH-exposed workers producing refractories (N=60, one-sided p=0.022), showed a significant association of *CYP1A1*2A* with the ratio of 1-OHP in the urine/air-borne pyrene. The ratio of 1-OHP level to the sum of ambient PAH was associated with the variation A114V in GSTP1 (p=0.02, two-sided exact Wilcoxon rank-sum test). Without consideration of personal ambient monitoring data the examined SNPs showed no statistically significant association with the biomonitoring data.

Due to the multiple statistical testing the reported associations between biomonitoring, personal ambient monitoring and genetic variants in genes coding for PAH metabolising enzymes have to be interpreted with caution and need to be reassessed in an extended molecular epidemiology study.

Introduction/Objective

Certain human biotransformation enzymes are involved in the activation and conjugation of polycyclic aromatic hydrocarbons (PAHs). These enzymes are controlled by polymorphic genes. The aim of this study was to investigate single nucleotide polymorphisms (SNPs) in the genes *CYP1A1*, *CYP1B1*, *CYP3A4*, *mEH*, *GSTM1*, *GSTT1*, and *GSTP1* by PCR-RFLP as well as real-time PCR (LightCycler™ technology) to analyse the influence of metabolic enzyme variants on biomarkers of exposure under consideration of external personal ambient monitoring data.

Methods

Biological monitoring markers (1-hydroxypyrene (1-OHP), hydroxy-phenanthrenes (OHPH)) were analysed by HPLC in post-shift urine samples (1,2) of 109 occupationally PAH-exposed male workers (production of refractories: n=60, graphite-electrode production: n=28, coke

oven: n=13, workers handling coal tar: n=8). The mean age was 37 years (range: 19-62 years). Smoking habits were assessed by both questionnaire and urinary cotinine. 72% of the workers were smokers. The control subjects (N=32) were also males but not occupationally exposed to PAH. The mean age was 46 years (range: 27-68 years). 41% were smokers as determined by urinary cotinine analysis. For all controls PAH metabolites in the urine were determined.

Prior to blood sampling, a questionnaire was completed for each worker during a personal interview by the company physician to elicit workplace description, smoking habits, medical history, age, diet and use of personal protection devices.

The study was approved by the ethic commission of the Ruhr-University Bochum and conducted in accordance with the definitions of the declaration of Helsinki.

Personal air sampling in the worker's breathing zone was carried out according to method 5506 published by the National Institute for Occupational Safety and Health (NIOSH) (3). The method was exactly used as described (4).

DNA was prepared from lymphocytes of frozen whole blood samples using the commercial PUREGENE® DNA isolation kit (Biozym, Hessisch-Oldendorf, Germany).

For the detection of SNPs in the genes *CYP1A1, CYP1B1,CYP3A4, mEH, GSTM1, GSTT1* and *GSTP1* established PCR and Real-time PCR protocols (5-14) were used.

Results

The PAH concentrations of total particulate PAH, air-borne pyrene and phenanthrene concentrations ranged between 0.97 and 1,848 µg/m^3 (median: 23.5 µg/m^3), 0.048 and 300.6 µg/m^3 (median: 1.48 µg/m^3) and 0.07 and 176.02 µg/m^3 (median: 4.24 µg/m^3) respectively. Concentrations of biomarkers ranged between 0.06 and 117.25 µmol/mol creatinine (1-OHP) as well as 0.69 and 71.11 µmol/mol creatinine (OHPH).

The analysis of PAH-relevant enzyme polymorphism on the ratio biological monitoring vs. ambient monitoring revealed that the ratio of the sum of urinary OHPH and the phenanthrene exposure, was significantly associated with the genetic variation g.3801T>C (*2A*) in *CYP1A1* (p=0.03; one-sided exact Wilcoxon rank-sum test). All PAH-exposed workers (N=109, one-sided p=0.05), and the subgroup of PAH-exposed workers producing refractories (N=60, one-sided p=0.022), showed a significant association of *CYP1A1*2A* with the ratio of 1-OHP in the urine vs. air-borne pyrene. The ratio of 1-OHP level to the sum of ambient PAH was associated with the variation A114V in GSTP1 (p=0.02, two-sided exact Wilcoxon rank-sum test). Without consideration of personal ambient monitoring data the examined SNPs showed no statistically significant association with the biological monitoring data.

Conclusions

Due to the multiple statistical testing the reported associations between biological monitoring, personal ambient monitoring and genetic variants in genes coding for PAH metabolising enzymes have to be reassessed in an extended molecular epidemiology study.

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