Influence of genetic polymorphisms on biomarkers of exposure and effects in children living in Upper Silesia

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This article is a follow-up to our previous molecular epidemiology studies on the DNA damage in children from the Upper Silesia region of Poland. It is expected that metabolic and DNA repair gene polymorphisms may modulate individual susceptibility to environmental exposure. In this study, we investigate the association between polymorphisms (CYP2D, EPHX1, GSTM1, GSTT1, GSTP1, NAT2) and DNA repair (XPD, XRCC1, XRCC3) genes and selected biomarkers of exposure and effect such as levels of 1-hydroxyxyprene (1-OHP) and urinary mutagenicity, aromatic DNA adducts, sister chromatid exchange (SCE) and micronuclei (MN) in 74 children. Both 1-OHP concentration and urinary mutagenicity tested by TA98+S9 were significantly higher in individuals with EPHX1 (exon 4) Arg/Arg genotype than in individuals with other genotype. The EPHX1 (exon 3) significantly affected urinary mutagenicity tested with strain YG1024+S9. The urinary mutagenicity in individuals with Tyr/Tyr homozygotes was lower than in individuals with His/His (1057±685 vs. 1432±1003 revertants/mol creatinine). XRCC3 Met/Met genotype was associated with significantly higher levels of 1-OHP in urine compared with only The/Met genotype. The PAH-DNA adduct levels in the subgroup with GSTM1 null genotype was 2-fold higher than in individuals with GSTM1 active (7.06±5.12 vs. 13.14±9.81 adduct/10⁸ nucleotides). The mean level of aromatic DNA adducts in children with deletion of the GSTT1 gene was significantly higher compared with individuals with that gene present (8.03±6.23 vs. 14.66±10.70 adduct/10⁸ nucleotides). Also the carriers of the XPD Lys/Lys genotype showed higher levels of DNA adducts than heterozygotes (13.16±9.70 vs. 6.81±5.86 adducts/10⁹ nucleotides). Children carrying the XRCC3-241 Met/Met genotype exhibited a higher number of SCE in peripheral blood lymphocytes than carriers of Thr/Met allele (8.15±0.86 vs. 7.62±0.79 SCE/cell). It was also observed that children with the GSTP1 slow conjugator had significantly elevated MN in peripheral blood lymphocytes compared with fast conjugator (4.23±3.49 vs. 6.56±5.00 MN/1000 cells).

Introduction

According to World Health Organisation, estimates up to 25% of the global burden of disease, especially in childhood, is due to preventable environmental exposure (1,2). There is a general recognition that children are more vulnerable to such exposure by virtue of their increased susceptibility and the higher doses received. An increased susceptibility in children is a consequence of higher levels of exposure and uptake, relative immaturity of metabolic and excretory pathways, and incomplete development of target organs (3,4). Although in recent years there has been an increased emphasis on research aimed at this specific susceptible population, there are still large gaps in the available data, especially in the area of chronic, low-level exposure of children in their home and school environment (5). Three main methods are used to assess human exposure to chemical and biologic agents: questionnaires and other indirect means, environmental monitoring including personal monitoring and biomonitoring. Recently, human biomonitoring has rapidly gained importance. Now, the measurement and the use of biomarkers of exposure, effects and susceptibility have replaced traditional environmental indicators (6).

A highly industrialised and densely populated region of Upper Silesia in Poland, characterised by considerable levels of occupational and environmental pollution followed by an increased damage of genetic material in the inhabitants, has been a major concern of Polish and cooperating foreign researchers. Starting from the early 90s of the 20th century, the interest has been focused mainly on molecular consequences of exposure to complex mixtures containing polycyclic aromatic hydrocarbons (PAHs) in coke-oven workers (7–11) and non-occupationally exposed inhabitants of Upper Silesia (12–18), including children (19–22).

The influence of individual genetic susceptibility on response to genotoxic agents is well documented in literature. The bio-activation of carcinogens is mediated by phase I enzymes and the reactive metabolites generated during the process are conjugated by phase II enzymes. Also several cellular repair pathways have evolved as defence mechanisms to maintain the genomic integrity against DNA damage (23–25).

Cytochrome P-450 monoxygenases (CYPs) are phase I enzymes that function in the metabolic activation of PAHs and other pre-carcinogens. Many CYP450 enzymes are polymorphic and genotypes associated with high enzyme activity or high inducibility are supposed to be at risk. Microsomal epoxide hydrolase (mEH) is a phase II enzyme, which catalyses the hydrolysis of epoxides into dihydrodiols. This reaction usually leads to detoxification but in the case of PAHs, mEH is part of a metabolic activation route, since the dihydrodiol can further be metabolised by CYP to highly reactive diol epoxides, such as benzo(a)pyrene diol epoxide (BPDE). One of the major groups of detoxifying enzymes is glutathione S-transferases (GSTs). Each GST has distinct catalytic properties: conjugation with glutathione, peroxidation and isomerisation. N-acetyl transferases (NATs) can catalyse both reactions of detoxification,
such as N-acetylation, and activation, such as O-acetylation of N-hydroxyl amines (26,27).

The *Xeroderma pigmentosum* (XP) genes, including the 5′→3′ helicase encoding *XPD* gene, code for enzymes that participate in nucleotide excision repair pathway. The central part in base excision repair plays the X-ray repair cross-complementation group 1 (*XRC1*) protein acting as a mediator of complex protein–protein interactions involving poly(ADP-ribose) polymerase, DNA polymerase I and DNA ligase III. The XRC3, in turn, is one of the RAD51-like proteins involved in homologous recombination to maintain chromosome stability and DNA damage repair. Many common single-nucleotide polymorphisms have been described in the *XPD, XRC1* and *XRCC3* genes. However, data on their functional significance and understanding of their impact on susceptibility to carcinogens are still inconsistent and insufficient (27–29).

In this study, we investigate the association between allelic variants of genes encoding for phase I enzymes (*CYP2D6* and *EPHX1*), phase II enzymes (*GSTM1, GSTP1, GSTT1, NAT2*) and DNA repair proteins (*XPD, XRC1, XRCC3*) and selected biomarkers of exposure and effect such as levels of 1-hydroxypyrene (1-OHP) and urinary mutagenicity, aromatic DNA adducts, sister chromatid exchange (SCE) and micronuclei (MN) in 74 children from Upper Silesia region of Poland. In our previous study carried out on this population, a significant influence of exposure to environmental agents on the induction of cytogenetic effects in peripheral blood lymphocytes (PBL) was observed (21).

**Materials and methods**

**Individuals**
The examined population included 74 healthy children (47 boys and 27 girls), aged 5–14 years, who lived in Katowice and Sosnowiec—two cities located in the most polluted region of the Silesian province. The study was approved by the Ethics Committee of the Silesian Medical Academy in Katowice. In compliance with the Polish law, parents of all children participating in this study gave their written consent. All parents completed a self-administered questionnaire form including items concerning children’s demographic variables, health status, medical treatment, dietary habits and socioeconomic conditions, etc. The study population consisted of the same children for whom the relationships between DNA adducts and SCE, as well as between lead and MN, in blood had already been studied before (21).

**Blood and urine sampling**

Blood sampling took place in the Institute of Occupational Medicine and Environmental Health in Sosnowiec during the cold season between November 1998 and March 1999. Blood samples were collected into EDTA-containing tubes. Genomic DNA was isolated immediately. DNA and urine samples were kept frozen at −80°C until analysis.

**Determination of 1-OHP in urine**

The determination of 1-OHP in urine was carried out using the high-pressure liquid chromatography method developed by Jongeneelen and Anzion (30). 1-OHP in the 20ml urine samples was enzymatically deconjugated and then transferred to primed C18 Octadecyl cartridges, washed with 10ml of water and eluted with 9ml of methanol. The components of the eluate were separated by high-pressure liquid chromatography on the HP 1090 apparatus (Hewlett Packard) with the ODS C18 column, whereas 1-OHP was quantitatively determined using the fluorescence detector HP 1046 (Hewlett Packard) with 229 nm excitation and 400 nm emission wavelengths. Concentrations of 1-OHP were expressed in millimole per mole creatinine to account for differences in urine dilution.

**Urinary mutagenicity**

The organic substances present in urine were condensed using adsorption and desorption methods on columns filled with organic resin XAD-2 (31). The extracts were examined by plate incorporation test (32). Before the examination, the acetone extracts of urine were dissolved in dimethyl sulfoxide. Three doses, 20, 40 and 80 μl, of dimethyl sulfoxide solution (representing 6, 12 and 24 ml of urine) were examined twice with *Salmonella typhimurium* strains: TA98 and YG1024 with metabolic activation (+S9 mixture). Mutagenic effect was expressed as a number of induced revertants per millimole of creatinine (10).

**DNA extraction**

DNA isolation was carried out using proteinase K digestion, phenol–chloroform extraction and ethanol precipitation as described in standard protocols. The concentration of DNA was determined spectrophotometrically by measuring the UV absorbance at 260 nm and the purity was ascertained by the ratio at 260/280 nm.

**Aromatic DNA adducts analysis**

DNA adducts were assessed using 32P-post-labelling method mainly described by Randerath and Randerath (33). DNA (5 μg) was digested with micrococcal nuclease, phosphodiesterase from calf spleen and nuclease P1 from *Penicillium citrinum*. Then the digested DNA was labelled with [γ-32P]ATP catalysed by T9 polynucleotide kinase. Redundant [γ-32P]ATP were eliminated by aprotase. The labelled material was then separated by thin layer chromatography on polyethyleneimine–cellulose plates and estimated by autoradiography. The diagonally radioactive zone was excised and quantified by scintillation counting. The parts of the plates without spots were used as background.

**Determination of cytogenetic endpoints in lymphocytes**

Cultures. Venous blood was taken from each subject using heparinised vacutainer tubes. Lymphocyte cultures were set up by adding 0.5 ml of heparinised blood to 4.5 ml of medium (RPMI 1640) supplemented with 20% heat-inactivated fetal bovine serum, antibiotics (penicillin and streptomycin) and 1-glutamine. Lymphocytes were stimulated by 1% phytohaemagglutinin.

**MN assay.** The cultures were incubated at 37°C for 72 and 44 h after the initiation of cultures; cytoschalasin B at a concentration of 6mg/ml was added to arrest cytokinesis. MN slides were stained with 10% Giemsa in phosphate buffer. A total of 1000 binucleated cells with well-preserved cytoplasm were examined for each subject on coded slides. The total number of MN and the frequency of binucleated cells with MN were scored (34).

**SCE assay.** The cultures were incubated for 72 h at 37°C with 0.25ml of 5-bromo-20-deoxyuridine. Colcemid was added 2 h before harvesting. The cells were collected by centrifugation, resuspended in a pre-warmed hypotonic solution (0.075 M KCl) for 20 min and fixed in acetic acid/methanol (1:3, v/v). Chromosome preparations and stained slides were prepared following the procedure by Anthosina and Porjadkowa (35). SCE was scored in 50 metaphases and presented as a number of SCE per cell. Data from <50 metaphases were discarded (36).

**Analysis of polymorphisms**

Blood samples were collected into EDTA-containing tubes. Genomic DNA was extracted from the peripheral lymphocytes by proteinase K digestion followed by standard protocols with phenol–chloroform extraction and ethanol precipitation.

The polymorphic deletions of *GSTM1* and *GSTM7* genes were determined by multiplex polymerase chain reaction (PCR) amplification (37). Allelic variants of the *CYP2D6, EPHX1, GSTP1, NAT2, XPD, XRC1* and *XRCC3* genes were identified by PCR-restriction fragment length polymorphism (RFLP) methods as described by others (38–44). Previously published multiplex PCR and PCR-RFLP protocols with some modifications were used. The amplification reactions were carried out in a total reaction volume of 30 μl containing 100ng genomic DNA, 1U Taq DNA Polymerase, 1x PCR buffer (10mM Tris-HCl, pH 8.3, 50mM KCl, 1.5mM MgCl 2 ), 0.2mM of each dNTP and 12.5–50.0 pmol of primers. PCR amplification was conducted in an Eppendorf Mastercycler gradient thermal cycler. The PCR and RFLP products were analysed in ethidium bromide–stained 1.8 and 2.5% agarose gels, respectively. All studied allelic variants, primer sequences and PCR-RFLP conditions are summarised in Table I.
Genetic polymorphisms, biomarkers, children

**Table I. Sequences of primers and restriction enzymes used in multiplex PCR and PCR-RFLP reactions**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer name</th>
<th>Primer sequence</th>
<th>Amplicon length (bp)</th>
<th>Annealing T&lt;sub&gt;a&lt;/sub&gt; (°C)</th>
<th>Restriction enzyme</th>
<th>Allele name (RFLP fragments length bp)</th>
</tr>
</thead>
</table>
| CYP2D6     | P51         | 5′-GCTGAGGCTCCATGACCT-3′ | 201 | 60 | BstAI | *1 (201)  
*3 (180; 121)  
*4 (190; 163) |
|            | P522        | 5′-GGGCTGTTCTCAGTACAT-3′ | 353 | 60 | MvaI | *1 (210)  
*3 (180; 121)  
*4 (190; 139; 24) |
| P*3        | 5′-CCCTTGGCAAGAGTCGTTG-3′ | 231 | 56 | Thl111 | T (209; 22)  
C (231)  
A (295; 62) |
| EPHX1      | EPHX3F      | 5′-CTGCTACCTCGTCCATCC-3′ | 132 | 52 | – | – |
| EPHX3R     | 5′-AATCTTCAGCTGGAAGTC-3′ | 357 | 59 | Rsal | G (174; 121) |
| GSTM1†     | J6          | 5′-GCTCATTGCTCTGAAAGGT-3′ | 280 | – | – | – |
| E7A        | 5′-TTGGAAGGCTCAAGGGG-3′ | 173 | 58 | Snab | *A (173)  
*B or C (142; 31)  
*C (170) |
| E7B        | 5′-TTGGAAGGGCAAGCAGG-3′ | 170 | 58 | BstUI | *A (170)  
*C (170) |
| BGI1       | 5′-CAACTTCATCCAGGTCC-3′ | 480 | 60 | – | – |
| BGI2       | 5′-GAGAGCGCAAGAGACCT-3′ | 800 | – | – | – |
| GSTP1      | P1F1        | 5′-CTGCTACCTCGTCCATCC-3′ | 1093 | 50 | KpnI | *4 (660; 433)  
*5B (1093) |
| P1R1       | 5′-CTGCTACCTCGTCCATCC-3′ | 344 | 61 | PstI | A (244; 110) |
| P1F2       | 5′-ACTGCTTCCATCATAAGGT-3′ | 434 | 60 | BclI | C (171; 110; 63)  
G (461; 278; 132)  
A (593; 278) |
| P1R2       | 5′-CTGCTTCCATCATAAGGT-3′ | 353 | 59 | Rsal | C (97; 39)  
T (136) |
| VDRF       | 5′-GCTGAGGCTCAAGGCTGTAAC-3′ | – | – | – | – |
| VDRR       | 5′-GCTGAGGCTCAAGGCTGTAAC-3′ | – | – | – | – |
| NAT2       | Hu7         | 5′-ATCCTAGAAGATCTCTCAGGAC-3′ | 1093 | 50 | KpnI | *4 (660; 433)  
*5B (1093) |
|            | Hu16        | 5′-GATGAAAGATTTGTGTAATTA-3′ | 344 | 61 | PstI | A (244; 110) |
| XPD        | CC5         | 5′-TCCAAATCCTGCTCTACTG-3′ | 353 | 60 | Rsal | C (97; 39)  
T (136) |
|            | CC4         | 5′-CTGGGCTTAAAGGCTGTTGG-3′ | 353 | 60 | Rsal | C (97; 39)  
T (136) |
| XRCC1      | CC3         | 5′-CATGCTGCTCACTACTACTG-3′ | 353 | 60 | Rsal | C (97; 39)  
T (136) |
|            | CC4         | 5′-ATCTGCTTCCATCATAAGGT-3′ | 353 | 60 | Rsal | C (97; 39)  
T (136) |
| XRCC3      | CC7         | 5′-GCTGAGGCTCAAGGCTGTAAC-3′ | 136 | 61 | NcoI | C (97; 39)  
T (136) |
|            | CC8         | 5′-GCTGAGGCTCAAGGCTGTAAC-3′ | 136 | 61 | NcoI | C (97; 39)  
T (136) |

†New England Biolabs, Germany.
*Fermentas UAB, Lithuania.
‡Deletion genotypes of GSTT1 and GSTM1 were determined by multiplex PCR.

**Statistical analysis**

Questionnaire and analytical data were stored in a database and statistically analysed using STATISTICA for Windows, Version 10, 2011 (StatSoft). Normal distribution was tested according to Shapiro–Wilks test. The levels of SCE were normally distributed. The distributions of 1-OHP concentration and urinary mutagenicity and the level of DNA adducts and MN were skewed to the right. Therefore, they were transformed (log or square root) to make their distribution normal (1-OHP, urinary mutagenicity and DNA adducts) or stabilise the variance (MN). The arithmetic mean and the standard deviation were used to describe the frequency distribution of biomarkers of exposure (1-OHP, urinary mutagenicity and DNA adducts) and effects (MN and SCE in peripheral blood lymphocytes). The differences between the groups were analysed using the Student’s t-test when the variance was equal or the Levene’s t-test when the variance was unequal. For the analysis of associations between the genotype distribution and other discrete parameters, chi-square test in contingency tables was used. A multiple linear regression analysis was applied to determine the effect of polymorphisms on biomarkers of exposure and effects with the confounding factors such as place of living, gender, environmental tobacco smoke and risk connected with the use of coal-fired stoves in houses.

**Results**

**Distribution of genotypes**

Out of the original group of 74 children for whom data on biomarkers of exposure to PAHs and biomarkers of effect were available (21), the GST (M1, M3, P1, T1), NAT2 and EPHX1 genotypes were determined in 72 individuals, whereas the CYP2D6, XPD and XRCC3 genotypes were determined in 71 individuals. The XRCC1 genotype distribution was successfully estimated in 69 children. Polymorphisms in the genes of phases I and II were merged into dichotomous variable based upon the known effect on enzymatic function (e.g. low and fast). For DNA repair genes, all three genotypes were analysed. Variant distribution of all studied polymorphic genes in the group is shown in Table II. The frequencies were similar to those observed in other healthy Caucasian populations and the distribution was in agreement with Hardy–Weinberg equilibrium. Girls had a significantly higher (or 2.4 time higher) frequency of the GSTM1 active genotype than boys (P = 0.026) and only three children living in a house with coal-fired stove had a CYP2D6 poor metaboliser genotype (P = 0.042).

**Influence of genotypes on biomarkers of exposure**

The influence of genetic polymorphisms of phase I and phase II enzymes and DNA repair on concentration of 1-OHP and urinary mutagenicity is shown in Table III. Both 1-OHP concentration (Figure 1) and urinary mutagenicity tested by TA98+S9 (Figure 2) were significantly higher in individuals (only three children) with EPHX1 (exon 4) Arg/
It was observed that urinary mutagenicity in individuals with Tyr/Tyr homozygotes was lower (activity) than in individuals with Tyr/His and His/His (activity) (1057 ± 685 vs. 1432 ± 1003 revertants/mol creatinine, respectively, P = 0.039). XRCC3 Met/Met genotype was associated with significantly higher levels of 1-OHP in urine compared with only The/Met genotypes (P = 0.010).
Genetic polymorphisms, biomarkers, children

Our previous study showed that the exposure to emission from indoor coal-fired stoves affected the level of 1-OHP and urinary mutagenicity in children. It was also noticed that there was a relationship between DNA adducts and SCE, as well as between lead and MN (all measured in blood). The level of DNA adducts and SCE was clearly and significantly associated with gender with higher levels found in girls (21).

Among the studied gene polymorphisms, only *EPHX1* and *XRCC3* had a significant effect on the tested markers of exposure to PAHs in Silesian children. It was observed that only children with genotype Arg/Arg in *EPHX1* (exon 4) excreted about twice as much of 1-OHP as children with His/His and His/Arg genotypes (Figure 1). The same phenomenon occurred in the case of the urinary mutagenicity tested by TA98+S9 (Figure 2). This could, however, be due to the fact that only three children had genotype Arg/Arg. Urinary mutagenicity levels tested with strain YG1024+S9 were statistically lower among Silesian children with the *EPHX1* Tyr/Tyr genotype (exon 3 polymorphism).

Fig. 1. The influence of genotype of *EPHX1* (exon 4) of children on the level of urinary 1-hydroxypyrene.

So far, the influence of the *EPHX1* gene polymorphisms at the level of markers of exposure has been studied only in association with occupational exposure. Several studies have indicated consistent associations between low mEH activity genotype/phenotype and lower levels of 1-OHP, urinary mutagenicity, DNA adducts and BPDE–serum albumin adducts (45–48). Results regarding the influence of both *EPHX1* polymorphisms and their combinations on cancer risk were inconsistent (49,50).

Microsomal epoxide hydrolase plays an important role in the detoxification of toxic, highly reactive intermediates formed by CYP-mediated reactions (51). Generally, investigations of the effect of *EPHX1* polymorphism focus on two polymorphic sites but the impact of these sequence variations on the enzyme activity is not yet conclusive (52,53). Furthermore, *EPHX1* variants seem not to have a strong impact on the enzyme kinetics, probably due to amino acid exchanges distant from the catalytic centre of the protein (46).

Fig. 2. The influence of genotype of *EPHX1* (exon 4) of children on the level of urinary mutagenicity in *Salmonella typhimurium* TA98 strain (with metabolic activation).

GSTs are one of the major groups of detoxifying enzymes. Each GST has distinct catalytic properties: conjugation with glutathione, peroxidation and isomerisation. Essential non-enzymatic role of GST connection with modulation of signalling pathways have also been shown (54). In our study, no effect of *GSTM1* gene polymorphism on the level of excreted 1-OHP was found, whereas in Mexican children with the *GSTM1* null polymorphism, the risk of high urinary 1-OHP concentrations was five times higher (55).

No influence of *GSTM1* polymorphism on urinary mutagenicity in children has been observed in our studies, which is similar to results obtained for non-smoking Polish women and Italian healthy smokers (16,56).

So far the significant impact on selected biomarkers of effect in children from Silesia has been attributed to polymorphism of genes such as *GSTM1*, *GSTT1*, *GSTP1*, *XPD* and *XRCC3*. The present study demonstrated a significant association between PAH-DNA adduct level and *GSTM1* and *GSTT1* polymorphism, alone or together (Table IV, Figure 3). There was a 3-fold higher number of DNA adducts in Silesian children with *GSTM1* and *GSTT1* deletions compared with those with both genes present (20.3 ± 10.35/10⁸ nucleotides and 7.2 ± 5.36/10⁸ nucleotides). The reliability of these results is limited due to the small number of children with deletion of two genes but this problem is extremely interesting because it can be assumed that a complete lack of both active enzymes is responsible for a fairly ineffective elimination of reactive xenobiotics derivatives.

Multiple linear regression analysis

Multiple linear regression analysis was carried out taking into consideration the following factors: place of living, gender, indoor exposure to environmental tobacco smoke, emission from indoor coal-fired stoves and genetic polymorphisms. The analysis showed that *EPHX1* exon 4 polymorphism influenced urinary mutagenicity tested by TA98+S9 in a subgroup of children living in houses (n = 4) with a coal-fired stove. The significant influence of *GSTM1* genotype on the level of DNA adducts was observed only in 13 girls. When the gene–gene interaction was examined, it was found out that only *GSTM1* and *GSTT1* combined genotypes influenced the DNA adduct levels. Children with deletions of both genes (n = 10) had significantly higher aromatic DNA adduct levels than those with the genes present (n = 35; Figure 3).

Discussion

Our previous study showed that the exposure to emission from indoor coal-fired stoves affected the level of 1-OHP and urinary mutagenicity in children. It was also noticed that there was a relationship between DNA adducts and SCE, as well as between lead and MN (all measured in blood). The level of DNA adducts and SCE was clearly and significantly associated with gender with higher levels found in girls (21).
**Table IV.** Biomarkers of effect in Silesian children by genotypes of phase I, phase II metabolic and DNA repair enzymes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Enzyme activity/ genotype</th>
<th>DNA adducts (per 10⁸ nucleotides)</th>
<th>SCE (per cell)</th>
<th>MN (per 1000 cells)</th>
</tr>
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<tr>
<td></td>
<td></td>
<td>N</td>
<td>Mean (standard deviation)</td>
<td>P</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>Extensive metaboliser</td>
<td>49</td>
<td>9.11 (6.42)</td>
<td>0.902</td>
</tr>
<tr>
<td></td>
<td>Poor metaboliser</td>
<td>22</td>
<td>10.40 (10.83)</td>
<td></td>
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<tr>
<td>EPHX1 (exon 3)</td>
<td>Low enzyme activity</td>
<td>28</td>
<td>9.68 (9.44)</td>
<td>0.696</td>
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<td></td>
<td>High enzyme activity</td>
<td>44</td>
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<tr>
<td>EPHX1 (exon 4)</td>
<td>Low enzyme activity</td>
<td>45</td>
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<td>0.905</td>
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<td></td>
<td>High enzyme activity</td>
<td>27</td>
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<td></td>
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<tr>
<td>GSTM1</td>
<td>Functional enzyme</td>
<td>42</td>
<td>7.06 (5.12)</td>
<td>0.005</td>
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<tr>
<td></td>
<td>Null activity</td>
<td>30</td>
<td>13.14 (9.81)</td>
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<tr>
<td>GSTT1</td>
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<td>GSTP1</td>
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<td>10.21 (8.54)</td>
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<td>XPD</td>
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<td>CC</td>
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**Fig. 3.** The influence of genotype of GSTM1 and GSTT1 on the level of DNA adducts in PBL in children.

GSTM1 is known to detoxify arene oxides, including the ultimate carcinogenic form of benzo(a)pyrene, BPDE, but it is not involved in the detoxification of aromatic amines. Some studies suggest that individuals who are homozygous null have an increased risk of cancer at a number of sites—lung, bladder, colon and breast (57). Individuals classified as GSTM1 null should theoretically have a higher level of DNA damage (e.g. PAH-DNA adducts) compared with individuals with the gene present. No effect of GSTM1 genotypes on lymphocyte DNA adducts (32P) was found in Italian (58), French (59) and Polish coke-oven workers (60). In environmentally exposed non-smoking women, DNA adducts were correlated with pollution levels only in GSTM1 active individuals (61). The results on Polish environmentally exposed individuals showed higher PAH-DNA adduct levels only in summer samples from subjects with GSTM1 null and in those with the GSTM1 null/GSTM1 Ile/Val combination (62). Wang et al. (63) studying PAH-DNA adduct formation in African American, Dominican, and Polish mothers and their newborns who were environmentally exposed to PAHs found that GSTM1-02 deletion in African Americans had a protective effect.

No influence of GSTM1, GSTT1, GSTP1 and NAT2 on DNA adduct levels has been reported in coke-oven workers from the Czech Republic (64), Netherland (65), Portugal (66) and Finland (45). Also two studies on women environmentally exposed to PAHs indicated a lack of influence of CYP1A1 and GSTM1 genotypes on DNA adducts (16,67). No significant effect of CYP1A1, GSTM1 and GSTT1 genotype on the levels of 1-OHP and DNA adducts in children attending schools in the inner city of Bangkok and rural areas were observed (68).

However, the results are ambiguous, which can be contributed to different methods for PAH-DNA adduct analyses using different targets, sources and/or levels of exposure (57). It may also attributed to the fact that the genes of the GST group among many enzymatic and non-enzymatic functions have may involved in maintenance of DNA stability and modulate DNA repair (54).

No papers have been found regarding the impact of GSTT1 null allele on the level of aromatic adducts in DNA or of additive effect of the combination GSTM1 null/GSTT1 null on this biomarker. The biological consequences of this polymorphism are fairly difficult to predict, as the enzyme is involved in both detoxification and activation reactions. Epidemiological studies do not show any clear association between the GSTT1 null genotype and cancer development but an additive effect of the combination GSTT1 null/GSTM1 null was observed for certain types of cancers (57).
**GSTP1** is another important GST responsible for metabolism of PAHs. The 105Val (allele A) variant should consequently be the risk factor for cancer in which PAH is involved in the aetiopathology due to a lower rate of detoxification of the active metabolite.

Our observations concerning the lower frequency of micronuclei in lymphocytes of children with wild-type homozgyous genotypes **GSTP1** (A/A) confirm the results of other studies. Similar results were obtained for population of the coke-oven workers (69) and 53 policemen from Prague (70) in which homozygotes with the **GSTP1** Val1105 allele had significantly higher MN frequencies than those with the Ile105 allele.

The impact of **GSTP1** polymorphism on the increase of DNA damage (such as micronuclei) was demonstrated in two works performed with PBLs *in vitro* exposed to styrene-7,8-oxide (SO) (71) and doxorubicin (72). In lymphocytes from healthy donors exposed to SO, a non-significant increase in induced MN frequency was found for A/B and A/C genotypes compared with the wild-type homozgyous genotype A/A (71). Lymphocytes from individuals with the homozgyous genotype for the variant (Val/Val) had a significantly higher number of doxorubicin-induced micronuclei than those with the heterozygous genotype. The occurrence of spontaneous lesions (background controls) in DNA was higher in lymphocytes from individuals with the Ile/Ile genotype compared with the heterozygotes (72) similarly to healthy male volunteers in the study of Czech policemen (70).

Up to now, the major research activities have been focused on polymorphisms in DNA repair genes as an important component of the individual susceptibility phenomenon because DNA repair activities are closely involved with the protection of the genome (73). The greater health impact is expected for genetic polymorphisms that affect genes with key functions in DNA repair such as e.g. **XPDL**, **XRCC1** or **XRCC3**. Some studies suggest that polymorphisms in these genes can modulate spontaneous and/or *in vitro*-induced DNA damage and/or cancer risk (27,74). However, the overall weight of such evidence is low because of the minimal incremental risk associated with variant alleles and inconsistencies in the published data (75).

In Silesian children, significantly higher level of PAH-DNA adducts was observed in the **XPDL** Lys/Lys homozygotes, in comparison with Lys/Gln heterozygotes (*P* = 0.002). A similar effect of **XPDL** polymorphism on DNA adducts was observed in the study of exposure to PAHs carried out among population from Prague, where homozgyous carriers of the Lys/Lys (AA) showed the highest level of total DNA adduct. This, however, was observed only in the control group and not among the exposed policemen (70). A similar effect of **XPDL** homozygote on chromosome aberration and micronuclei, as observed in our study, was recorded in 31 Caucasian women from the USA and 291 volunteers from Slovakia (76,77). However, in the case of traffic workers, never-smoking healthy individuals, officers from the Municipality of Rome and people exposed to Prestige oil with at least one variant allele for **XPDL** Lys751Gln polymorphism showed increased mean levels of DNA adducts, SCE and MN frequency (78–80). A possible explanation for these results is that amino acid variants in different domains of **XPDL** may not only affect different protein activities, resulting in the expression of different phenotypes (81), but also the **XPDL** codon 751 polymorphism may have divergent effects in different DNA repair pathways. Moreover, this substitution could be in linkage with another **XPD** variant responsible for phenotypic effect. It is possible that different alleles may be in linkage disequilibrium with responsible **XPD** variant in different populations.

The Thr241Met substitution in **XRCC3** is a non-conservative change with a possible biological implication for functionality of the enzyme and/or the interaction with other protein involved in DNA damage repair (82). **XRCC3** gene polymorphism had a significant impact on the level of urinary excretion of 1-OHP and the level of SCE in PBL of the Silesian children. Children who are homozygous Met/Met excreted significantly more 1-OHP and had a greater number of SCE in peripheral blood lymphocytes in comparison with homozygous Thr/Thr. No studies on the impact of **XRCC3** polymorphism on excretion of urinary 1-OHP and SCE levels have been found, either. The **XRCC3**-241Met variant was closely associated with higher DNA adduct level (42). In some studies, it was demonstrated that the level of SCE was influenced by **XRCCI** gene polymorphism but not by **XRCC3** (83–85). In this study, a clear association between the **XRCC3**-241Met variant and SCE in children’s PBL suggested the contribution of homologous recombination pathways in SCE formation.

Numerous studies have investigated the effects of polymorphisms in genes involved in activation/detoxification of carcinogens and DNA repair on phenotypic biomarkers of exposure and effect. Only three studies have been conducted in environmentally exposed children (55,68,86). Since our findings are based on relatively small numbers of children, and therefore might be accidental, further studies are required to confirm our observations and to obtain explanations for the suggested influence of metabolism and, especially DNA repair, gene polymorphisms on biomarkers of exposure and effect.

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