**Null Results in Brief**

**No Effect of CYP1B1 Val432Leu Polymorphism on CYP1B1 Messenger RNA Levels in an Organochlorine-Exposed Population in Slovakia**

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**Introduction**

Cytochrome P450 1B1 (CYP1B1) is a phase I enzyme involved in the metabolic activation of many polycyclic aromatic hydrocarbons. It is also involved in the hydroxylation of estradiol to 4-hydroxyestradiol, a potentially genotoxic metabolite that is suggested to play a role in carcinogenesis. CYP1B1 is expressed in many tissues including mononuclear peripheral blood cells and is regulated through the aryl hydrocarbon receptor–mediated pathway, which can be induced by several environmental chemicals, including polycyclic aromatic hydrocarbons and persistent organochlorine pollutants such as polychlorinated biphenyls (PCB). A single nucleotide polymorphism of the CYP1B1 gene (1,294G→C) leads to an amino acid substitution in codon 432 (Val to Leu). This substitution results in a lower catalytic activity of the enzyme (1), but the effect of this CYP1B1 Val432Leu polymorphism on CYP1B1 gene expression and its inducibility is not clear. Gene expression and genotype of CYP1B1 can easily be determined in blood lymphocytes, making it a potential candidate to be used as biomarker for exposure to aryl hydrocarbon receptor agonists. We studied the correlation between CYP1B1 gene expression level and exposure to environmental factors within the CYP1B1 Val432Leu genotype groups. This study was part of a European project (PCBRISK) that investigates a human population exposed to environmental pollution as a consequence of the 25-year-long production of PCBs in eastern Slovakia.

**Study Population and Methods**

Blood samples were taken from two populations in two different areas in Slovakia, the Michalovce District (polluted area) and the Stropkov District (less polluted, reference area). RNA was isolated from the lymphocytes and the samples (n = 334) were stored at −80°C until analysis. Methods for blood collection, isolation of human lymphocytes, and RNA isolation are described elsewhere (2). Blood concentrations of PCBs were measured as sum of all PCBs or as nanograms of toxic equivalent (TEQ) per kilogram of lipid, which was defined as the sum of PCBs normalized with the WHO toxic equivalent factors. CYP1B1 genotype was determined by a PCR-RFLP method using 750 ng RNA, adapted after Tang et al. (3). cDNA (4.5 µg; Fermentas, Inc., Hanover, MD) was used as positive control for endonuclease restriction. CYP1B1 mRNA expression was normalized by a log10 transformation. Deviations from the Hardy-Weinberg equilibrium were calculated using the two-sided χ2 test. One-way ANOVA was conducted and Pearson correlation coefficients were calculated to study associations between variables. Statistical calculations were done using GraphPad InStat 3.06 (GraphPad Software, Inc., San Diego, CA) and SAS (SAS Institute, Inc., Cary, NC).

**Results and Discussion**

Of the 334 RNA samples stored, we were able to determine the CYP1B1 Val432Leu genotype of 114 samples (34%). Of the other samples, RNA concentrations were too low for genotyping. Of one sample no information on gender was available. In the total population, the allele frequency for Leu was 0.44 (Table 1). This allele frequency is similar to other observed frequencies in various healthy Caucasian populations and did not statistically significantly deviate from the Hardy-Weinberg equilibrium (P = 0.1). Genotype frequencies were not different within the male or female subgroups and they did not deviate from the Hardy-Weinberg equilibrium (male P = 0.1 and female P = 0.5).

No difference in total PCB levels and TEQs was observed between the populations from the polluted area and the reference area (data not shown) and therefore the populations were regarded as one, equally exposed, population. Within this entire population, there was no significant correlation between PCB levels or TEQs in blood lipid and CYP1B1 mRNA levels (R = 0.09, P = 0.4, n = 114 and R = 0.01, P = 0.9, n = 114, respectively). When we divided the entire population in the Val/Val, Val/Leu, and Leu/Leu genotype groups, no statistically significant differences were found in CYP1B1 mRNA levels, PCB levels, or TEQs between the genotypes (Table 1). There were no significant correlations between PCB levels in blood lipid and CYP1B1 expression levels with correlations of 0.18 (Val/Val, P = 0.28, n = 40), 0.11 (Val/Leu, P = 0.51, n = 38), and −0.17 (Leu/Leu, P = 0.43, n = 22). Further, no statistically significant associations between CYP1B1 expression and PCB levels or TEQs in blood lipids were found using linear regression modeling with adjustment for smoking and gender, two possible confounding factors for CYP1B1 expression (data not shown).
Our data indicate that environmental exposure to PCBs in our study population had no statistically significant effect on mRNA expression of CYP1B1, regardless of the CYP1B1 Val432Leu genotype. This is inconsistent with the study described by Hanaoka et al. (4). They found a higher expression level of CYP1B1 in peripheral blood lymphocytes on exposure to polycyclic aromatic hydrocarbons in Chinese coke oven workers who had at least one CYP1B1 432Leu allele. However, the number of subjects in that study was small (37 cases and 13 control workers) and it concerned an Asian population. Two other studies reported a correlation between CYP1B1 expression levels in peripheral blood lymphocytes and exposure to aryl hydrocarbon receptor agonists. One found a correlation with dioxin exposure higher than 6.5 pg TEQs/g lipid in a Japanese population (5); the other found no effect of polycyclic aromatic hydrocarbon exposure in healthy volunteers of unknown ethnicity (6). Spencer et al. (7) have suggested using CYP1B1 expression as a biomarker for in vivo exposure to dioxin-like compounds. However, despite the high blood levels of PCBs and TEQs in our study population and the separation of the population into genetically more homogenous groups with respect to CYP1B1 Val432Leu genotype, no correlation was observed between PCB levels and CYP1B1 mRNA levels in human lymphocytes. In addition, the large interindividual variability in CYP1B1 expression and PCB blood levels in the population makes it unlikely that possible, subtle effects of PCB exposure on RNA expression can be detected. This makes the suitability to use CYP1B1 expression as biomarker for exposure questionable.

### Acknowledgments

We thank Tomas Trnovec and Jan Petrick who supplied the blood samples and Rob Beelen for his help with the statistical analyses.

### References

2. Cantón RF, Besselink HT, Sanderson JT, Bobchuyver S, Brouwer B, van den Berg M. Expression of CYP1A1 and 1B1 mRNA in blood lymphocytes from two district populations in Slovakia compared to total TEQs in blood as measured by the DRE-CALUX® assay. Organohalogen Compounds 2003;64:215–8.

### Table 1. Characteristics of the Slovakian study population

<table>
<thead>
<tr>
<th></th>
<th>Val/Val*</th>
<th>Val/Leu</th>
<th>Leu/Leu</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCB level (ng sum PCB/kg blood lipid)</td>
<td>2,907.1 ± 6,684.2</td>
<td>1,768.6 ± 2,329.0</td>
<td>2,629.1 ± 5,121.0</td>
<td>0.59</td>
</tr>
<tr>
<td>TEQ (ng/kg blood lipid)</td>
<td>88.6 ± 36.2</td>
<td>86.9 ± 64.3</td>
<td>82.1 ± 30.9</td>
<td>0.86</td>
</tr>
<tr>
<td>CYP1B1 expression (arbitrary units)</td>
<td>0.61 ± 2.49</td>
<td>0.96 ± 2.17</td>
<td>0.44 ± 3.56</td>
<td>0.72</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>25/17</td>
<td>23/20</td>
<td>17/11</td>
<td>0.54</td>
</tr>
<tr>
<td>Smoking status (smoker/unknown)</td>
<td>28/14</td>
<td>22/22</td>
<td>18/10</td>
<td>0.40</td>
</tr>
<tr>
<td>Number of subjects (% of total)</td>
<td>42 (36.8%)</td>
<td>44 (38.6%)</td>
<td>28 (24.6%)</td>
<td></td>
</tr>
</tbody>
</table>

NOTE: Values are represented as mean ± SD or, in case of gender and smoking status, as number of individuals.

*CYP1B1 Val432Leu genotype.
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