Susceptibility to urinary bladder cancer: relevance of rs9642880[T], GSTM1 0/0 and occupational exposure

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Recently, a genome-wide single nucleotide polymorphism association study has identified a sequence variant 30 kb upstream of the c-Myc gene (allele T of rs9642880) that confers susceptibility to bladder cancer. However, the role of exposure to bladder carcinogens has not been considered. This prompted us to analyse the relevance of this polymorphism in 515 bladder cancer cases and 893 controls where the quality and quantity of occupational exposure to bladder carcinogens has been documented.

When we analysed a hospital-based case–control series not selected for occupational exposure, rs9642880[T] was not influential but GSTM1 0/0 was significantly associated with bladder cancer risk. Therefore, the degree to which rs9642880[T] and GSTM1 0/0 confer susceptibility to urinary bladder cancer seems to depend on the extent of exposure to urinary bladder carcinogens. Pharmacogenetics and Genomics 19:903–906 © 2009 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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We first analysed rs9642880 in 212 hospital-based patients with bladder cancer and 194 controls from the same hospital who did not suffer from cancer (Wittenberg case–control series; supplemental Patients and Methods, supplemental digital content 1, http://links.lww.com/FPC/A60). Approximately 34% of the bladder cancer cases were homozygous for the rs9642880 variant and their risk of developing urinary bladder cancer was 1.77-fold that of noncarriers with population attributable risk (PAR) of 14% (Wittenberg case–control series, Table 1, supplemental digital content 2, http://links.lww.com/FPC/A61). The association was also significant after adjusting the analysis to take into account age, sex and smoking habits [odds ratio (OR) = 1.697; P = 0.030; supplemental Table 1, supplemental digital content 3, http://links.lww.com/FPC/A62]. These are very similar results to those of Kiemeney et al. [1], who obtained an increased risk of approximately 1.5-fold for individuals homozygous for the rs9642880 variant and a PAR of 17%. Similar to Kiemeney et al. [1], we also observed a significant association for the homozygous variant, whereas the heterozygous genotype seemed to be less influential (supplemental Table 1, supplemental digital content 3, http://links.lww.com/FPC/A62). Our Wittenberg case–control series resembles five of seven case–control groups of Kiemeney et al. [1] that also represent hospital-based case–control series of bladder cancer patients.

Our second case series consisted of 216 bladder cancer patients consecutively evaluated for the legal compensation of this malignancy as an occupational disease (in Germany: BK 1301), resulting from exposure to occupational bladder carcinogens, which were mostly carcinogenic aromatic amines, azo dyes based on carcinogenic aromatic amines or polycyclic aromatic hydrocarbons (PAHs) (Table 2; supplemental Patients and Methods, supplemental digital content 1, http://links.lww.com/FPC/A60). The association was also significant after adjusting the analysis to take into account age, sex and smoking habits [odds ratio (OR) = 1.697; P = 0.030; supplemental Table 1, supplemental digital content 3, http://links.lww.com/FPC/A62]. These are very similar results to those of Kiemeney et al. [1], who obtained an increased risk of approximately 1.5-fold for individuals homozygous for the rs9642880 variant and a PAR of 17%. Similar to Kiemeney et al. [1], we also observed a significant association for the homozygous variant, whereas the heterozygous genotype seemed to be less influential (supplemental Table 1, supplemental digital content 3, http://links.lww.com/FPC/A62). Our Wittenberg case–control series resembles five of seven case–control groups of Kiemeney et al. [1] that also represent hospital-based case–control series of bladder cancer patients.
supplemental digital content 1, http://links.lww.com/FPC/A63 (Occupational case–control series). The 699 controls were from the same geographical region within Germany, for whom occupational histories are not available. Considering these case numbers, the study has a power of 96%, 66% or 38% to detect an OR of 1.5, 1.3 and 1.2, respectively, assuming a minor allele frequency of 0.5. Despite the relative high power (for detection of ORs of 1.3 or higher), we could not detect an association between variant rs9642880 and bladder cancer in the Wittenberg but neither in the occupational nor in the Dortmund case–control series. In contrast, GSTM1 0/0 was associated with increased bladder cancer risk in the occupational and in the Dortmund but not in the Wittenberg case series. The single nucleotide polymorphism rs9642880 is in Hardy–Weinberg equilibrium in controls as well as in cases in all study groups. Sex was not associated with any of the polymorphisms in any of the study groups. The result of a multivariate logistic regression, adjusted for cigarette smoking, age and sex, is shown in supplemental Table 1. No significant influence of GSTT1 and GSTP1 was observed in the three case–control series (supplemental Table 4, http://links.lww.com/FPC/A69).

We recruited a third group of patients, the so-called ‘Dortmund case series’, comprised of patients from an area with a previous prevalence of coal, iron and steel industries, which were associated with exposures to PAHs (Table 2; supplemental Patients and Methods, supplemental digital content 1, http://links.lww.com/FPC/A63). As before, we did not observe an influence of rs9642880[T] (OR = 1.038; P = 0.872), whereas GSTM1 0/0 was associated with an increased risk of bladder cancer (OR = 2.431; P < 0.001, Table 1; adjusted for age, sex and smoking habits: OR = 2.669; P < 0.001, supplemental Table 3, supplemental digital content 4, http://links.lww.com/FPC/A63).

The lack of significance of GSTM1 in the Wittenberg case–control series should be discussed with caution, because GSTM1 0/0 is higher in the Wittenberg (0.53) compared with the Dortmund controls (0.48). In principle, the GSTM1 0/0 frequencies in our controls are in agreement with previous studies. In European studies, GSTM1 is usually absent in approximately 50% of the control population with small variations similar to our present study. Garte et al. [2] report a frequency of 51.6% of GSTM1 0/0 in Germany (n = 734) and, for instance, frequencies of 53.6% in Denmark, 54% in France, 49.4% in Italy and 50.4% in the Netherlands. Considering the German controls of Garte et al. [2] as controls for our case series, there was no significant deviation in the proportion of the GSTM1 genotypes in the Wittenberg cases (χ² test of equal proportions P = 0.0831) and in the occupational cases (P = 0.2163), but again there was deviation for the Dortmund cases (P = 0.0012). However, from the statistical point of view, it is difficult to use cases and controls from completely different areas, such as Dortmund and Wittenberg that are located in West Germany and in the

### Table 1: Association of the carriage of the rs9642880[T] allele as well as GSTM1 0/0 with urinary bladder cancer

<table>
<thead>
<tr>
<th>Study population (No. of cases/No. of controls)</th>
<th>rs9642880[T]</th>
<th>GSTM1 0/0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency</td>
<td>Frequency</td>
<td>Frequency</td>
</tr>
<tr>
<td>Wittenberg case–control series (212/194)</td>
<td>0.56 0.48</td>
<td>0.58 0.53</td>
</tr>
<tr>
<td>Occupational case–control series (216/699)</td>
<td>0.51 0.49</td>
<td>0.56 0.48</td>
</tr>
<tr>
<td>Dortmund case–control series (87/699)</td>
<td>0.49 0.49</td>
<td>0.69 0.48</td>
</tr>
</tbody>
</table>

The variant rs9642880 conferred susceptibility to bladder cancer in the Wittenberg but neither in the occupational nor in the Dortmund case–control series. In contrast, GSTM1 0/0 was associated with increased bladder cancer risk in the occupational and in the Dortmund but not in the Wittenberg case series. The single nucleotide polymorphism rs9642880 is in Hardy–Weinberg equilibrium in controls as well as in cases in all study groups. Sex was not associated with any of the polymorphisms in any of the study groups. The result of a multivariate logistic regression, adjusted for cigarette smoking, age and sex, is shown in supplemental Table 1. No significant influence of GSTT1 and GSTP1 was observed in the three case–control series (supplemental Table 4, http://links.lww.com/FPC/A69).

CI, confidence interval; OR, odds ratio.

*Adjusted for age, cigarette smoking and sex the P value is 0.017 (supplemental Table 3, http://links.lww.com/FPC/A63).

### Table 2: The different influence of the rs9642880[T] and GSTM1 0/0 polymorphisms may be explained by different exposure scenarios

<table>
<thead>
<tr>
<th>Study population</th>
<th>Aromatic amines</th>
<th>Polycyclic aromatic hydrocarbons</th>
<th>Azo dyes*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wittenberg cases</td>
<td>13 (6)</td>
<td>18 (8)</td>
<td>36 (17)</td>
</tr>
<tr>
<td>Wittenberg, controls</td>
<td>2 (1)</td>
<td>2 (1)</td>
<td>22 (12)</td>
</tr>
<tr>
<td>Occupational case–control series, cases</td>
<td>132 (61)</td>
<td>59 (27)</td>
<td>134 (62)</td>
</tr>
<tr>
<td>Dortmund case–control series, cases</td>
<td>0 (0)</td>
<td>45 (52)</td>
<td>7 (8)</td>
</tr>
</tbody>
</table>

The Wittenberg case–control series is a hospital-based group of patients in which only a relatively small fraction of individuals were occupationally exposed to bladder carcinogens. In contrast, high fractions of individuals from the occupational case series, representing patients evaluated for bladder cancer as an occupational disease, and from the Dortmund case series, were occupationally exposed to bladder carcinogens. Patients’ characteristics are given in supplemental Tables 2–4. The difference between Wittenberg cases and controls was significant for aromatic amines (P = 0.0064) and polycyclic aromatic hydrocarbons (P < 0.001) but not for azo dyes (P = 0.090) in the χ² test. All differences between the Wittenberg cases and the occupational as well as the Dortmund cases were significant (P < 0.05).

*Azo dyes based on carcinogenic aromatic amines.
former German Democratic Republic, respectively. Interestingly, in a large bladder cancer study performed in Berlin, which is about 100 km from the Wittenberg study area, 52% of the 374 hospital-based controls were of the GSTM1 0/0 genotype (cases: 58%) [3]. Furthermore, in a case series of 170 unselected newborns, the GSTM1 0/0 percentage was 54% [4]. This is close to the 51.6% reported by Garte et al. [2], so that a systematic bias of the Wittenberg controls seems to be less likely. We compared the relevance of rs9642880[T] and GSTM1 using a logistic regression model, including only these two parameters. This comparison indicates that rs9642880[T] is relevant only in the Wittenberg series (P = 0.0308, Wald test) but not in the two other study groups (occupational: P = 0.3895; Dortmund: P = 0.9978), whereas GSTM1 is not significant in Wittenberg (P = 0.1748) but is significant in the occupational series (P = 0.0052) and in the Dortmund series (P < 0.001). Expressed as different PAR of GSTM1 and rs9642880[T] in the three study groups, in the Wittenberg study, GSTM1 0/0 has a PAR of 9%, whereas the PAR in the occupational and in the Dortmund study groups are 21% and 54%, respectively. PAR of rs9642880[T] was lower: 14% in the Wittenberg study group, 8% in the occupational series and 3% in the Dortmund series.

The association between GSTM1 0/0 and bladder cancer has been extensively studied. Large meta-analyses have shown that GSTM1 null status is associated with a modest increase in the risk of bladder cancer [2,5,6]. The degree to which GSTM1 0/0 confers susceptibility to bladder cancer was related to smoking habits. The OR of GSTM1 0/0 was 2.37 [95% confidence interval (CI): 1.80–3.12] in ‘ever smokers’ compared with only 1.20 (95% CI: 0.86–1.66) in ‘never smokers’ [5], suggesting that GSTM1 is particularly relevant for individuals exposed to high levels of carcinogens, which are substrates of GSTM1, such as highly reactive metabolites of PAHs. The occupational exposure of the Dortmund bladder cancer patient series as well as the frequency of GSTM1 0/0 bladder cancer patients (69%) is high. It is the only study group [7] that was excluded from the meta-analysis of Engel et al. [5] because of the occupational exposure, presenting 75% GSTM1 0/0 bladder cancer cases in occupations from the coal, iron and steel industries (n = 44) compared with 64% in all other occupations (n = 45). The study of Hung et al. [8] performed in a highly industrialized Italian area reported a comparable frequency of 66% GSTM1 0/0 in 201 bladder cancer patients. The OR was 1.69 (95% CI: 1.11–2.56). In an analysis of interaction of GSTM1 genotype and occupational exposure to PAHs, the OR was 1.73 (95% CI: 1.03–2.90) for ‘never’ and 1.92 (95% CI: 1.06–3.45) for ‘ever’ exposed GSTM1 0/0 bladder cancer patients; for aromatic amines the OR was 1.61 (95% CI: 1.04–2.48) for ‘never’ and 2.77 (95% CI: 1.08–7.10) for ‘ever’ exposed bladder cancer patients [8].

In conclusion, we have confirmed the observation of Kiemeneij et al. [1] that variant rs9642880 confers susceptibility to urinary bladder cancer in hospital-based case–control series, if specific occupational exposure to bladder carcinogens is rare. In addition, we show that bladder cancer that occurs as a result of occupational exposure to aromatic amines or PAHs may lead to a different situation, where polymorphisms of detoxifying enzymes, such as GSTM1, become more relevant. As the fraction of occupationally exposed individuals in the general population of bladder cancer patients is usually low [9], it is plausible that in most hospital-based or population-based case–control series, the influence of rs9642880 will dominate, whereas that of GSTM1 is less relevant. Our observations may also explain the heterogeneity of results in the follow-up groups of the study of Kiemeneij et al. [1] where rs9642880[T] was clearly associated with urinary bladder cancer in the combined follow-up groups (P = 7.98 × 10⁻⁷), whereas no statistically significant influence was seen in some of the individual follow-up groups. An example is the follow-up group from Italy-Brescia, where rs9642880[T] was not significant (P = 0.419). It has recently been published that occupational exposure to PAHs in the patients from Italy-Brescia was unusually high (39% of the cases were occupationally exposed [10]) and that GSTM1 0/0 was associated with an increased risk of bladder cancer in this case–control series [8]. It should be considered that even in the follow-up groups of Kiemeneij et al. [1], where rs9642880[T] was not significant, such as the group from Italy-Brescia, the OR was still higher than 1. Therefore, it cannot be excluded that rs9642880 is still relevant. However, together with our case–control series, where exposure to bladder carcinogens has precisely been documented, there is evidence that in the presence of occupational bladder carcinogens, which are substrates of GSTM1, the relevance of GSTM1 increases whereas rs9642880 becomes less relevant. This is in line with the finding of Kiemeneij et al. [1], who observed no differences in the frequencies of rs9642880 between ever-smoking (i.e. private exposure to PAHs) and never-smoking bladder cancer patients. Although these results are interesting, they are preliminary and should be subject to further investigations in larger studies.

The eligibility criteria for occupational disability compensations are restrictive in most countries. Additional criteria that help in identifying cases where past occupational exposure has contributed to carcinogenesis would be highly welcome. In cases, where the patient’s occupational exposure is in a grey area, which would justify a recognition just as well as a rejection of the claim, the genetic background for bladder cancer would be an additional argument in the decision process. Thus, in the future it will be interesting to analyse whether genome-wide data on SNPs and copy number variations will allow an establishment of algorithms differentiating
between bladder carcinomas with occupational exposure and without occupational exposure to PAHs or aromatic amines.

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