Genetic polymorphisms of MPO, COMT, MnSOD, NQO1, interactions with environmental exposures and bladder cancer risk

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Abbreviations: COMT, catechol-O-methyltransferase; HWE, Hardy–Weinberg Equilibrium; MnSOD, manganese superoxide dismutase; MPO, myeloperoxidase; NQO1, NAD(P)H:quinone oxidoreductase; PAH, polycyclic aromatic hydrocarbon.

Introduction

Tobacco smoking and occupational exposure are major risk factors of bladder cancer via exposure to polycyclic aromatic hydrocarbons (PAHs) and aromatic amines (1,2). The exposure to these carcinogens may play an important role in bladder carcinogenesis.

Oxidative stress in vivo might be modulated by the enzymes such as myeloperoxidase (MPO), catechol-O-methyltransferase (COMT), manganese superoxide dismutase (MnSOD), and NAD(P)H:quinone oxidoreductase (NQO1). MPO is a lysosomal enzyme located in neutrophils and monocytes, and it produces a strong oxidant, hypochlorous acid, for microbicidal activity (6). Through the release of reactive oxygen species, MPO also activates procarcinogens in tobacco smoke, such as benzo[a]pyrene (7–9). A single G→C mutation transition in MPO was identified at the SP1 binding site, which might modify the carcinogen metabolism (10). A allele is associated with reduced mRNA expression and its transcription activity is ~25 times lower than G allele in vitro because of reduced binding of SP1 (10). There have been several epidemiological studies concerning MPO G→C polymorphism and lung cancer risk (11–16), which associated the variant to a reduced risk; however, no data on bladder cancer risk has been reported so far.

COMT catalyzes the methylation of various endobiotic and xenobiotic substances preventing quinone formation and redox cycling, and therefore might protect DNA from oxidative damage (17,18). A G to A transition, which results in amino acid change from valine to methionine at codon 108, leads to a lower COMT activity in a co-dominant manner. The enzyme activity of the Met/Met genotype is a quarter of that of the wild Met genotype, and subjects heterozygous exhibit intermediate enzyme activity (19). So far, there are only limited data on the effect of COMT polymorphisms in carcinogenesis (20–22).

Manganese superoxide dismutase (MnSOD) catalyzes the dismutation of superoxide radicals in mitochondria by converting anion superoxide into hydrogen peroxide and oxygen. It can be induced by free radical challenge and cigarette smoke, and plays a key role in protecting cells from oxidative stress (23–25). A C to T substitution was identified in MnSOD, which results in an amino acid change from alanine to valine at position 29 (25,26). The literature of the functional significance of the polymorphism and its relation to cancer risk has not been conclusive (25–29).

NQO1 is an important flavoenzyme in xenobiotic metabolism. It protects cells from oxidative damage by catalyzing the

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two-electron reduction of carcinogenic quinoid compound to their reduced form, such as hydroquinones (30). The 690C>T single nucleotide polymorphisms, which leads to replacement of proline to serine at codon 187, has been associated with lower enzyme activity as compared with wild-type (31–33).

It is hypothesized that the risk of bladder cancer can be influenced by polymorphisms in the genes that modulate oxidative stress in particular by interacting with relevant environmental exposures. The hypotheses were that polymorphism in the MPO gene confers a reduced risk of bladder cancer, while that of the other genes results in an increased risk. We therefore conducted a hospital-based case-control study among men in Brescia, Northern Italy, to investigate the effect of these polymorphisms on the bladder cancer risk. We were also intrigued by the possible gene–environment interactions with occupational exposures. There are no data available so far on the above genetic polymorphisms and occupational exposures to PAH and aromatic amines. We therefore tested the hypothesis that the effects of PAH and aromatic amines on bladder cancer risk are modified by the polymorphisms in genes that modulate oxidative stress. The detailed analysis of tobacco and occupational exposure will be discussed in a separate paper (Porru et al., in preparation).

Materials and methods

Study population and epidemiological data collection

This hospital-based case-control study was conducted in Brescia, a highly industrialized area in Northern Italy. Eligible subjects were male residents in the province, aged 20–80 years old. The cases were newly diagnosed bladder cancer patients in the urology departments of two main hospitals of Brescia with histological confirmation of the disease. The controls were patients admitted to the same hospitals during the same period of time, with urological non-neoplastic diseases, including hydronephrosis, urethral atresia, non-neoplastic urological diseases, prostatic adenoma, and hypertrophia, urological traumas, orchiepididymitis, hydrocele and unspecified urinary symptoms. Controls were frequency matched to cases on age (±5 years). From July 1997 to December 2000, there were 216 incident bladder cancer cases and 220 hospital controls eligible for the study. The response rate was 93% in cases and 97% in controls, leading to a total of 201 cases and 214 controls recruited in the study. A written informed consent was obtained from each recruited subject and the study was approved by the local Ethical Committee.

Cases and controls went through a face-to-face interview during hospital admission using standard questionnaires. Epidemiological data were collected on demographic variables, active and passive smoking history, liquid consumption and diet habits via a food-frequency questionnaire. Lifestyle occupational history was collected including each job lasting at least 1 year. Job titles and plant activity were coded according to the International Standard Classification of Occupation (ISCO, International Labor Office, 1968) and International Standard Classification of all Industrial Activities (ISIC, United Nations, 1968). For each specific job title, occupational physicians assessed blindly whether the subject was exposed to PAH or aromatic amines based on the information available, including plant activity, type of production, exposures to chemicals, detailed description of workplaces, job tasks, use of personal protective devices and hygienic behaviors in workplaces. Information provided by records on risk assessments included environmental monitoring data, health and safety company consultants, personal knowledge of local industries and literature data. When a job title was considered as related to exposure of PAH or aromatic amines, utilizing the methodology described in the previous publications (34–36), occupational physicians then further classified the exposure with four indices: (i) mode of contact: respiratory only, dermal only or both respiratory and dermal contact; (ii) frequency of the exposure: low as 1–5% of the working time, medium as 5.1–30% or high as >30% of the working time; (iii) level of the exposure: low, medium or high; (iv) reliability of the exposure evaluation, i.e. the confidence that the exposure level actually occurred, at three-level scale: possible, probable or definite, which was based on the personal judgment of the occupational physician, for each individual case, using a variety of information as mentioned above. More detailed information regarding occupational exposure assessment is available upon request.

Biological sample and genotyping

Blood samples were collected from all the subjects during the hospital admission. We prepared DNA solutions from whole blood as described by Spurr et al. (37). The DNA solution was cooled and stored at −30°C. The G–463A polymorphism in the promoter region of MPO was analyzed as described in the study by London et al. (38). The valine to methionine transition at amino acid 108 of C609T polymorphism in the MPO promoter region of MPO to G to A polymorphism, was examined using an Amplification Refractory Mutation System assay as described by Hoda et al. (39). The MnSOD C1137T polymorphism determining Ala-9Val amino acid change was analyzed using PCR–RFLP analysis as described by Ambrosone et al. (27). The proline to serine change at amino acid 187 of NQO1 due to C609T polymorphism was analyzed using a method based on PCR and RFLP analysis as described by Bartsch et al. (40). RFLP protocols were tested on a series of subjects with the known genotype in order to check the reliability and reproducibility of the assay. All uncertain results were re-analyzed with the same technique and usually only one more assay was sufficient to clarify any doubts. All analyses were conducted in one lab by technicians who were blind to case-control status.

Statistical analysis

The frequency distribution of demographic variables and putative risk factors of bladder cancer, including age, education and smoking was examined for cases and controls. The distribution of education differed slightly between cases and controls (P-value = 0.14).

Dietary items were quantified based on intake frequencies: weights of 0.1, 0.5, 1.5, 3.5, 5.5, 7, 17.5 and 30 were used for the categories of never or less than once per month, 1–3 times per month, 1–2 times per week, 3–4 times per week, 5–6 times per week, once per day, 2–3 times per day, >4 times per day, respectively. Total fruit and total vegetable intake were calculated as the sum of the weights of all the relevant food items. They were then dichotomized into high and low consumption based on the median among the controls, which were 18.05 for the fruit intake and 12.6 for the vegetable intake.

Smoking status was categorized into never smokers, light smokers and heavy smokers; the cut-off point between the last two categories was based on the median of cumulative smoking exposure among controls (26 packyear). Former smokers were smokers who had quitted smoking for at least 1 year. Exposures to PAH and aromatic amines were dichotomized into ever and never based on the occupational history. The detailed analyses utilizing the other exposure indices will be presented somewhere else (Porru et al., in preparation).

Hardy–Weinberg Equilibrium (HWE) was tested separately for cases and controls for each polymorphism; the allele frequency was calculated and the observed genotype frequency was compared with expected frequency using χ2 test. The effects of genetic polymorphisms on the risk of bladder cancer were estimated with odds ratio (OR) and its 95% confidence interval (CI), which were derived from unconditional multivariate logistic regression using STATA software. Based on the distribution of our data and the prior knowledge of bladder cancer, we included age, education and smoking packyear as potential confounders in our regression models. The main effects of polymorphism were first assessed separately for each genotype by creating dummy variables; genotypes were subsequently grouped based on either prior biological knowledge or the frequency distribution. As a result of the lack of complete information on the functional significance of MnSOD Ala-9Val polymorphism, we have analyzed the data under the assumption of both dominant (grouping heterozygous with homozygous rare allele) and recessive model (grouping heterozygous with wild-type).

In addition to the main effects of each polymorphism, we stratified study subjects according to smoking status to assess the differences between the genetic effects in different groups. Effect modifications of dietary antioxidants and occupational exposures to PAHs and aromatic amines were also evaluated. Dummy variables were created to estimate the effect of each combination of genotype and exposure status. Carriers of the protective allele without exposure to the environmental factor comprised reference group. Departure from multiplicative model of interaction was estimated. Since PAHs and aromatic amines are both present in tobacco smoke, the gene–occupation interaction analyses were further adjusted for smoking duration (years), average smoking intensity (no. cigarettes/day), time since quitting and current smoking status (yes/no/no).

Results

Table 1 shows the frequency distribution of the demographic variables and putative risk factors of bladder cancer. Around half of the cases were older than 65 years; and controls were slightly more educated than cases. As expected the smoking prevalence in cases was higher than in controls.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cases</th>
<th>Controls</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
In the control group, the allele frequency of MPO G-463A, COMT Val108Met, MnSOD Ala-9Val and NQO1 Pro187Ser polymorphism were 0.25, 0.47, 0.49 and 0.21, respectively. The allele distributions for the latter three polymorphisms in controls were under HWE with P-value of 0.31, 0.26 and 0.21, respectively, whereas the P-value for the MPO G-463A polymorphism was 0.002, mainly due to excess of homozygous rare allele in the population.

Table I shows the effect of MPO G-463A, COMT Val108Met, MnSOD Ala-9Val and NQO1 Pro187Ser polymorphism on bladder cancer risk. The estimates adjusted for age, smoking and occupational carcinogens were very similar to the crude estimates (results not shown). There was a protective effect of MPO A/A genotype on the risk of bladder cancer, with OR = 0.31 (95% CI = 0.12–0.80), and this effect was stronger in heavy smokers (OR = 0.12, 95% CI = 0.04–0.44). However, there seemed to be an inverse effect for MPO G/A genotype. We did not observe an association between COMT Val108Met polymorphism and risk of bladder cancer. The OR for the MnSOD Ala-9Val heterozygous genotype (Ala/Val) was very close to unity, which is in line with the assumption of the recessive model. We observed a positive association between MnSOD Val/Val genotype and bladder cancer risk with an OR of 1.91 (95% CI = 1.20–3.04), which seemed to be present only in the smokers. Compared with non-smoking subjects carrying the MnSOD Ala/Ala, Ala/Val genotypes, the OR for subjects who ever smoked and carried Val/Val genotype was 7.20 (95% CI = 2.33–16.1), suggesting a combined effect, although the P-value of interaction was not significant (P = 0.74). A marginal positive effect of NQO1 Pro187Ser polymorphism on bladder cancer risk was present (OR = 1.32, 95% CI = 0.87–2.00), which seemed to be more important in never smokers.

The results from interaction analyses of genetic factors and occupational exposures are shown in Table III. We observed an interaction between MnSOD Val/Val genotype and occupational exposure to PAH with a combined OR of 3.02 (95% CI = 1.35–6.74), and there seemed to be a combined effect of COMT Val108Met Val/Val genotype and exposure to aromatic amines, however the number is small. There was no interaction found between fruit and vegetable intake and any polymorphism (data not shown).

### Discussion

To our knowledge, this is one of the first epidemiological studies that evaluated the main effects of the MPO, COMT,
**Table III. Interaction of genetic factors and occupational exposures of PAH or aromatic amines**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Genotype</th>
<th>Occupational exposures</th>
<th>PAH</th>
<th>Aromatic amines</th>
<th>OR</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control no.</td>
<td>OR</td>
<td>95% CI</td>
<td>P-value</td>
<td>Control no.</td>
<td>OR</td>
</tr>
<tr>
<td><strong>MPO</strong></td>
<td>G/A, A/A</td>
<td>Never</td>
<td>53</td>
<td>58</td>
<td>1.53 (0.79, 2.97)</td>
<td>0.36</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>G/A, A/A</td>
<td>Ever</td>
<td>38</td>
<td>27</td>
<td>1.86 (1.0, 3.42)</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>G/G</td>
<td>Never</td>
<td>71</td>
<td>83</td>
<td>1.02 (0.60, 1.72)</td>
<td>103</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>G/G</td>
<td>Ever</td>
<td>39</td>
<td>46</td>
<td>1.04 (0.56, 1.91)</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td><strong>COMT</strong></td>
<td>Val/Val</td>
<td>Never</td>
<td>42</td>
<td>36</td>
<td>1.00 (0.95, 1.05)</td>
<td>0.38</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>Val/Val</td>
<td>Ever</td>
<td>20</td>
<td>21</td>
<td>0.90 (0.40, 2.03)</td>
<td>2</td>
<td>4.27 (0.83, 22.1)</td>
</tr>
<tr>
<td></td>
<td>Val/Met, Met/Met</td>
<td>Never</td>
<td>82</td>
<td>105</td>
<td>0.73 (0.41, 1.29)</td>
<td>130</td>
<td>147</td>
</tr>
<tr>
<td></td>
<td>Val/Met, Met/Met</td>
<td>Ever</td>
<td>57</td>
<td>52</td>
<td>1.01 (0.54, 1.90)</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td><strong>MnSOD</strong></td>
<td>Ala/Ala, Ala/Val</td>
<td>Never</td>
<td>82</td>
<td>109</td>
<td>1.70 (0.95, 3.06)</td>
<td>0.37</td>
<td>119</td>
</tr>
<tr>
<td></td>
<td>Ala/Ala, Ala/Val</td>
<td>Ever</td>
<td>51</td>
<td>60</td>
<td>1.11 (0.66, 1.84)</td>
<td>14</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Val/Val</td>
<td>Never</td>
<td>42</td>
<td>32</td>
<td>1.70 (0.95, 3.06)</td>
<td>64</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>Val/Val</td>
<td>Ever</td>
<td>26</td>
<td>13</td>
<td>3.02 (1.35, 6.74)</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td><strong>NQO1</strong></td>
<td>Pro/Pro</td>
<td>Never</td>
<td>71</td>
<td>88</td>
<td>1.00 (0.95, 1.05)</td>
<td>0.69</td>
<td>104</td>
</tr>
<tr>
<td></td>
<td>Pro/Pro</td>
<td>Ever</td>
<td>42</td>
<td>47</td>
<td>1.13 (0.64, 1.98)</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Pro/Ser, Ser/Ser</td>
<td>Never</td>
<td>53</td>
<td>53</td>
<td>1.23 (0.73, 2.10)</td>
<td>79</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>Pro/Ser, Ser/Ser</td>
<td>Ever</td>
<td>35</td>
<td>26</td>
<td>1.67 (0.88, 3.16)</td>
<td>9</td>
<td>7</td>
</tr>
</tbody>
</table>

OR, odds ratio adjusted for age, education and smoking duration (years), average smoking intensity (no. cig/day), time since quitting and current smoking status (yes versus no). P-value, P-value of interaction.

MnSOD and NQO1 polymorphisms on bladder cancer risk, and their potential interaction with environmental exposures including smoking, occupational exposures to PAH and aromatic amines. One of the strengths of this study is the detailed information on the occupational history, which enable an investigation of interaction between occupational exposures and genetic susceptibility.

Comparing with the previous studies of bladder cancer, this study has a sufficient sample size, which can detect a minimum OR of 1.7 for common polymorphism, for example COMT Val108Met, which has allele frequency of 0.47; and minimum OR of 1.8 for relatively rare polymorphisms, for example MPO G-463A, which has allele frequency of 0.25. The power of interaction analyses was relatively low. Take PAH exposure as an example, we have a reasonable sample size to detect a minimum interaction OR of 3.1 with MnSOD (whose prevalence of risk genotype is 0.21 among the controls).

Besides the limitations inherited from the hospital-based design, false positive results arising from multiple comparison is another potential limitation in this study. The number of false positive associations increases along with the number of associations tested. This problem can be addressed by increasing the sample size, by specifying a priori (which hypothesis will be tested) or by conducting more sophisticated statistical analysis such as hierarchical modeling.

In this study, we found a protective effect of MPO A/A genotype on bladder cancer risk, which agreed with our prior knowledge and expectation. However, the fact that the allele frequency of this polymorphism among controls is not under HWE may indicate that the control group was not selected randomly in terms of the distribution of this polymorphism, and suggests a possible selection bias in the controls. MPO plays an important role in microbicidal activity, and its activity is induced mainly by infection. Controls in this hospital-based case-control study are the patients with non-neoplastic urological diseases, whose occurrence might be associated with MPO genotype. This may also provide an explanation of the observation of opposite effects of heterozygous and homozygous rare allele. Nevertheless, the fact that the allele distribution among cases was not under HWE either (P-value = 0.06), and the increasing importance of MPO genotype along with the exposure to carcinogens, smoking in particular, are consistent with a role of this polymorphism in bladder cancer. When we recalculated the OR assuming controls were under HWE (using the expected frequency for control group), the crude OR remained in the same direction but the confidence interval widened (e.g. OR for A/A among heavy smokers = 0.33, 95% CI = 0.07–1.38).

We found that MnSOD Val/Val homozygous increased the risk of bladder cancer and the association was restricted to smokers, particularly heavy smokers. The interaction with smoking was more than multiplicative. Although the functional significance of this polymorphism is unclear, from a Chou Fasman analysis, −9Ala allele was predicted to form a typical amphilical helical structure, which is essential for its effective transport into mitochondria, and −9Val allele was predicted to disrupt this structure (26). Rosenblum and colleagues also suggested that the change of protein structure might influence the cellular allocation of the enzyme and transport of MnSOD into the mitochondria (25). Our findings support the hypothesis that the predicted structural change might result in the altered ROS scavenger activity in the mitochondria leading to a positive association between MnSOD Val/Val genotype and bladder cancer risk. Despite the low power of interaction analysis, we also observed an interaction between Val/Val genotype and occupational exposures to PAH, which is consistent with the previous hypothesis. The MnSOD polymorphism was only identified relatively recently. There is a clear need for further functional studies on how the polymorphism affects the cellular allocation of the enzyme and transport rate into mitochondria.

NQO1 converts the highly genotoxic benzoquinine to the less toxic hydroxy metabolites (32), and its Pro187Ser polymorphism has been shown to be associated with cancer risk, mainly of the lung. However, the epidemiological evidence is not consistent, and there is only limited information on bladder cancer so far. A population-based case-control study has shown that an NQO1 polymorphism increased the risk of urological malignancy (renal cell carcinoma and urothelial
carcinoma) (41), while a study from Italy showed no association between DNA adduct in white blood cells and \textit{NQO1} polymorphisms (42). In our study, we observed that the effect of \textit{NQO1} Prol875Ser polymorphism might be more important in never smokers. The available evidence regarding effect of \textit{NQO1} polymorphisms modified by smoking on tobacco-related cancer risk is not yet consistent (43–46). Our results agree with the hypothesis that the effects of certain metabolic genes might be more prominent in the low-level exposure to carcinogens, because the enzyme activity of rapid and slow metabolizers might both be saturated when one is heavily exposed to carcinogens (47). A large hospital-based case-control study has also shown a differential genetic susceptibility to lung cancer based on smoking behavior, where \textit{NQO1} polymorphisms seemly play a more important role when the exposure to carcinogen is moderate, although the results on never smokers were not reported (48). Our conclusion was confined by the sample size, and the possibility of chance finding cannot be excluded. Larger studies are needed to confirm this finding.

\textbf{COMT Val108Met} polymorphism did not appear to have an effect on bladder cancer risk. The joint OR for \textit{COMT} Val/Val genotype and occupational exposures to aromatic amines is seemly high; however, the precision of our estimate is threatened by small numbers. The same limitation applies to the high point estimate for the combination of MPO variants and exposure to aromatic amines.

It is important to see the effects of the genes modulating oxidative stress along with dietary antioxidants, and we did attempt to examine the interaction between genes and dietary factors. However, our data of dietary habits was relatively simplified and were not able to provide sufficient information.

In conclusion, these findings suggest that reactive oxygen species may play an important role in the bladder carcinogenesis, and individual susceptibility of bladder cancer may be modulated by \textit{MPO}, and \textit{MnSOD} polymorphisms. In addition, the combination of genetic factors and environmental exposures are likely to play an important role in bladder carcinogenesis.

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References


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