Distinct SNP Combinations Confer Susceptibility to Urinary Bladder Cancer in Smokers and Non-Smokers

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Abstract

Recently, genome-wide association studies have identified and validated genetic variations associated with urinary bladder cancer (UBC). However, it is still unknown whether the high-risk alleles of several SNPs interact with one another, leading to an even higher disease risk. Additionally, there is no information available on how the UBC risk due to these SNPs compare to the risk of cigarette smoking and to occupational exposure to urinary bladder carcinogens, and whether the same or different SNP combinations are relevant in smokers and non-smokers. To address these questions, we analyzed the genotypes of six SNPs, previously found to be associated with UBC, together with the GSTM1 deletion, in 1,595 UBC cases and 1,760 controls, stratified for smoking habits. We identified the strongest interactions of different orders and tested the stability of their effect by bootstrapping. We found that different SNP combinations were relevant in smokers and non-smokers. In smokers, polymorphisms involved in detoxification of cigarette smoke carcinogens were most relevant (GSTM1, rs11892031), in contrast to those in non-smokers with MYC and APOBEC3A near polymorphisms (rs9642880, rs1014971) being the most influential. Stable combinations of up to three high-risk alleles resulted in higher odds ratios (OR) than the individual SNPs, although the interaction effect was less than additive. The highest stable combination effects resulted in an OR of about 2.0, which is still lower than the ORs of cigarette smoking (here, current smokers' OR: 3.28) and comparable to occupational carcinogen exposure risks which, depending on the workplace, show mostly ORs up to 2.0.

Introduction

Urinary bladder cancer (UBC) is the ninth most common cancer worldwide [1]. The strongest known risk factors include cigarette smoking, occupational exposure to urinary bladder carcinogens, and male gender. It is well established that a deletion variant of the detoxifying phase II metabolizing enzyme glutathione S-transferase M1 (GSTM1), in addition to N-acetyltransferase 2 (NAT2) slow acetylation are associated with increased urinary bladder cancer risk [2–6]. Recently, further genetic variants have been identified and validated in several genome-wide association studies [7–12] and were extended to occupational exposure [13–15].

The recently discovered SNPs and the corresponding genes have already been comprehensively discussed [1]. Briefly, rs1014971 maps to a non-genic region of chromosome 2q37.1 [9] close to CBX6 and APOBEC3A. Chromobox homolog 7 (CBX7) positively regulates E-cadherin expression by interacting with histone deacetylase 2 [16]. This possibly explains why loss of CBX7 expression is associated with a highly malignant phenotype of carcinomas. Overexpression of APOBEC3 genes may lead to genetic instability [17]. Rs11892031 is located on chromosome 2q37 in an intronic region of the UDP-glucuronosyltransferase 1A (UGT1A) locus. UGT1A is a phase II metabolizing enzyme that catalyzes the glucuronidation and elimination of numerous xenobiotics [18,19]. Rs1495741 (on chromosome 8p22) is known as a tagging SNP of N-acetyltransferase 2 (NAT2) that distinguishes between fast and slow acetylators [20,21]. Compared to fast acetylators, slow acetylators have an increased bladder cancer risk, probably because of their decreased ability to efficiently detoxify aromatic amines. Rs710521[A] on chromosome 3q28 close to TP63 is associated with urinary bladder cancer risk [7,14]. TP63 shows strong homology to the tumour suppressor P53 [22,23; review: 1]. Rs8102137 on 19q12 maps to Cyclin E (CCNE1) which controls cell cycle progression at the G1/S transition [24; review: 1]. Rs9642889, 30 kb upstream of the MYC gene on chromosome 8q24.21, confers susceptibility to bladder cancer and influences expression of MYC [7,13]. The well-known proto oncogene MYC is involved in the control of proliferation and cell cycle progression [25]. Deletion of the detoxifying phase II enzyme glutathione S-transferase M1 (GSTM1) on chromosome 1q13.3 leads to a decreased detoxification of numerous xenobiotics, including
polycyclic aromatic hydrocarbons that are known bladder carcinogens [13,26]. Although the association of each of these SNPs with urinary bladder cancer risk has been validated and confirmed in several independent cohorts, it is still not known if there is an interaction among the high-risk alleles, and if their influence differs between smokers and non-smokers. Therefore, we determined the most influential genetic variants (rs1014971, rs11892031, rs1495741, rs710521, rs8102137, rs9642880, and GSTM1) in 1,595 bladder cancer cases and 1,760 controls. We performed interaction analyses addressing the following questions: Are there specific and stable SNP interactions resulting in higher odds ratios than individual SNPs? If so, are these SNP combinations identical or distinct between smokers and non-smokers? Finally, how high is the combined genetic (SNP-based) risk compared to that of cigarette smoking and occupational exposure? We report that specific SNP combinations show a higher UBC risk than individual SNPs, where distinct SNP combinations confer susceptibility in smokers and non-smokers. These risks are, however, still small when compared to that of cigarette smoking.

Materials and Methods

Ethics Statement

The sample collection by the Leibniz Research Centre for Working Environment and Human Factors (IfADo) was approved by the ethics commission of the Leibniz Research Centre for Working Environment and Human Factors (Ethikkommission des Leibniz-Instituts für Arbeitsforschung an der TU Dortmund) and the institutional review board of the Leibniz Research Centre for Working Environment and Human Factors (Wissenschaftlicher Beirat des Leibniz-Instituts für Arbeitsforschung an der TU Dortmund). All participants provided their written informed consent.

Patients

To investigate whether there is a combined effect of SNPs associated with UBC, a total of 1,595 UBC cases of European descent and 1,760 controls of European descent from four case-control series collected by the Leibniz Research Centre for Working Environment and Human Factors (IfADo) were genotyped at the glutathione S-transferase M1 (GSTM1) and six SNPs (rs1014971, rs11892031, rs1495741, rs710521, rs8102137, rs9642880) previously identified in genome-wide association studies to be associated with UBC [7,9].

This data set comprised confirmed urinary bladder cancer cases and controls without malignant disease from the Department of Urology, Semmelweis University, Budapest, Hungary (“Hungary”); 246 cases and 78 controls), the Department of Urology, Paul Gerhardt Foundation, Lutherstadt Wittenberg, Germany (“East Germany”); 218 cases and 213 controls), the “West Germany – Ongoing” case-control series conducted at five hospitals (in total, 646 cases and 525 controls), and the “West Germany – Industrial” burdened case-control series (in total, 485 cases – 111 UBC cases from the Department of Urology, Klinikum Dortmund, Germany, and 374 UBC cases surveyed for recognition of an occupational disease – and 944 controls). Information on profession obtained by questionnaire was available for the “East Germany” case-control series only (information on profession: 216 cases and 211 controls) [27,28]. Detailed descriptions of these four case-control series can be found in [15].

Patients’ characteristics, such as distribution of gender, age at diagnosis for cases and age at examination for controls, as well as numbers of cases and controls in the individual case-control series, are summarized in Tables S1, S2, and S3. 101 cases and 37 controls with unknown smoking habits were excluded from the interaction analysis in the study groups, leading to a total of 1,494 cases and 1,723 controls that were finally considered to determine the impact of SNP combinations on the UBC risk.

Polymorphisms

Isolation of genomic DNA of leukocytes was performed according to standard procedures. Genotypes of the SNPs rs1014971, rs11892031, rs1495741, rs710521, rs8102137, and rs9642880 were detected by TaqMan® Assay. Details of the SNPs are given in Appendix S1 and Table S4.

The homozygous GSTM1 deletion was detected by the amplification of the GSTM1 DNA sequence segment with 218 base pairs by means of PCR [29,30]. After gel-electrophoresis using ethidium bromide, the DNA product was detected using UV light. This method helped determine whether at least one copy of the GSTM1 gene was present or totally missing.

Statistical Analysis

Cigarette smoking was defined as non-smokers, former smokers, i.e. smokers that quit smoking at least one year before diagnosis (cases) or examination (controls), and current smokers. Former and current smokers were pooled together as “ever smokers”. Analyses were performed stratified for non-smokers, former smokers and current smokers as well as for ever smokers. Analyses on the combined ever smokers groups reflect the past exposure to bladder carcinogens accounting for the latency time of bladder cancer of several decades. Age was defined as “age at diagnosis” for the cases and “age at examination” for the control persons.

Deviations from Hardy-Weinberg equilibrium (HWE) were checked in each study group and separately for cases and controls using χ² tests (for the results, see Table S5). Associations of polymorphisms and smoking habits with UBC were evaluated applying χ² tests, odds ratios (OR), and 95% confidence intervals (95% CI). Moreover, ORs and 95% CIs adjusted for age, gender, smoking habits, and study site were estimated using logistic regression.

The ORs of the individual polymorphisms, and combinations of these polymorphisms in the total cohort as well as in subgroups defined by the smoking status of the subjects, were determined by considering the dominant and recessive effects of the SNPs. For each interaction of p polymorphisms (p = 2, ..., 7), the ten combinations showing the OR with the lowest p-values were identified in each of the subgroups. To check whether it is appropriate to compute p-values for higher-order SNP interactions based on a χ² distribution with one degree of freedom, we also determined permutation p-values and compared these with the parametric p-values. Additionally, a bootstrap strategy was used to investigate the stability of the ORs of the SNP combinations of different sizes in the subgroups. To achieve this, 500 bootstrap samples were drawn from the respective subgroup and counted to determine how often the top 10 SNP combinations from the original analysis appeared among the top 10, top 20, and top 50 SNP combinations of the same number of SNPs from the analyses of the corresponding 500 bootstrap samples. To test whether the OR of a certain SNP combination differs between the ever smokers and the non-smokers, logistic regression models were fitted containing parameters for the respective SNP combination, smoking status, and the interaction between these two factors. The standard test for the interaction parameter in this logistic regression model was used to test whether the ORs differ significantly between smokers and non-smokers. Details on this and other statistical analyses can be found in Appendix S2.
Figure 1. Optimal odds ratios for combinations of one to seven polymorphisms. For the computation of the optimal odds ratios (OR), all possible combinations of one to seven of the polymorphisms rs1014971, rs9642880, rs710521, rs8102137, rs11892031, rs1495741 and GSTM1 were considered. (A) Profile plots for the odds ratios in the total group (black line) and the subgroups of ever smokers (red line), current smokers (green), former smokers (blue) and non-smokers (cyan). The lines were included for clarity of information and not to suggest a continuous development. Dashed lines indicate when number of cases and/or number of controls fall below 100. In these situations, the corresponding odds ratios should be interpreted with caution. (B)–(F): For the optimal combinations shown in (A), box plots of odds ratios computed in 500 bootstrap samples from (B) the total group, (C) the ever smokers, (D) the current smokers, (E) the former smokers and (F) the non-smokers. In twelve of the bootstrap samples (all but one in the analyses of the seven-way interactions in the total and the smoker group), the odds ratios were larger than 15. For a better presentation, these odds ratios are not displayed in the corresponding box plots. The crosses mark the odds ratios of the optimal combinations in the original analysis. The corresponding plots of the test statistics are shown in Figure S1.

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Population attributable risks (PAR) indicating the proportion of cases that could be attributed to a certain risk factor, and combined PARs for two or more independent risk factors were calculated according to [31]. The PARs of the individual polymorphism were calculated based on adjusted and unadjusted ORs. Combined PARs were determined based on the adjusted ORs of the homozygous and heterozygous vs. the reference genotypes of each SNP. ORs were adjusted for age, gender, smoking habits, study site (in case of combined study groups) and all measured polymorphisms but rs11892031, as this SNP has a rather protective effect in about 16% of the population of European descent [32]. All four study groups were used to determine the PAR due to smoking habits and genetic risk factors in the present study, whereas the PAR for certain professions was based on the “East Germany” case-control series only.

For an overview of UBC risk factors from the literature, we performed an extensive literature search using PubMed. We included the relevant papers on UBC causes in populations of European descent. If possible, we used the given adjusted ORs to determine the PAR from published studies. Otherwise, unadjusted ORs or ORs calculated from the published frequencies were used. Estimation of ORs of combined genetic risk factors was done for varying frequencies assuming a PAR of 30%.

Results

Analysis of ORs of SNP Combinations

Currently, it is unknown whether genetic variants associated with increased UBC risk interact with one another resulting in higher odds ratios (OR) for combinations than for individual SNPs. Therefore, we analyzed the ORs from combinations of up to seven polymorphisms that were previously found to be individually associated with UBC [2,7,9,20]. The ORs as well as the corresponding 95% confidence intervals (95% CI) and p-values for the individual SNPs, determined in the analysis of our total study group and subgroups defined by the smoking habits, are summarized in Table S6.

Analyzing the SNP combinations, the ORs of the optimal SNP combinations, in general, increased with the numbers of combined SNPs (Figure 1A). However, case numbers of the high-risk alleles decreased rapidly when several SNPs were combined, thus leading to relatively high variability of the odds ratios in the bootstrap sample (Figure 1B–F). Here the variation typically increased with decreasing number of subjects. In contrast to the ORs, the Wald statistics corresponding to the ORs increased from individual SNPs to combinations of three polymorphisms. However, no further increase was observed (Figure S1), which is again due to high variances and small sample sizes.

In Tables 1, 2, 3 and 4, the ORs with 95% CIs and the p-values of the ten combinations of two and three polymorphisms with the smallest p-values found in the analysis of the ever smokers and the non-smokers are shown. The ORs of the top ten individual effects as well as the top ten two-way and three-way interactions in the total group and in the smoker subgroups are presented in Tables S7, S8, S9, S10, S11, S12, S13, S14, S15, S16, S17, S18, S19, S20 and S21. Additionally, we summarized how often the seven polymorphisms occur in the top ten two-way and three-way interactions in the different subgroups (Table 5).

Table 1. Top ten two-way interactions found in the analysis of the ever smokers.

| SNP combination | OR (95% CI) | P-value |
|-----------------|-------------|---------|
| rs11892031 [A/A] × GSTM1 null | 1.48 (1.25–1.76) | 0.0024 |
| rs8102137 [C/T, T/T] × GSTM1 null | 1.51 (1.25–1.82) | 0.0040 |
| rs710521 [A/A, A/G] × GSTM1 null | 1.46 (1.22–1.73) | 0.0062 |
| rs710521 [A/A, A/G] × GSTM1 present | 0.69 (0.58–0.83) | 0.0105 |
| rs9642880 [G/G, G/T] × GSTM1 present | 0.69 (0.57–0.82) | 0.0113 |
| rs11892031 [A/A, A/C] × GSTM1 present | 0.70 (0.59–0.84) | 0.0185 |
| rs11892031 [A/A, A/C] × GSTM1 null | 1.42 (1.19–1.69) | 0.0204 |
| rs1014971 [C/C, C/T] × GSTM1 present | 0.71 (0.60–0.84) | 0.0303 |
| rs1014971 [A/A, A/G] × GSTM1 null | 1.40 (1.18–1.66) | 0.0398 |
| rs1014971 [C/C, C/T] × GSTM1 null | 1.38 (1.16–1.64) | 0.0703 |

Table 2. Top ten two-way interactions found in the analysis of the non-smokers.

| SNP combination | OR (95% CI) | P-value |
|-----------------|-------------|---------|
| rs9642880 [G/T, T/T] × rs1014971 [C/C] | 1.91 (1.44–2.51) | 0.0015 |
| rs9642880 [G/G, G/T] × rs1014971 [C/T, T/T] | 0.56 (0.43–0.74) | 0.0112 |
| rs710521 [A/A, A/G] × rs1014971 [C/C] | 1.68 (1.28–2.20) | 0.0458 |
| rs1014971 [C/C] × rs1495741 [A/A, A/G] | 1.66 (1.27–2.16) | 0.0524 |
| rs1014971 [C/C] × rs11892031 [A/A, A/C] | 1.65 (1.27–2.16) | 0.0564 |
| rs1014971 [C/T, T/T] × rs8102137 [C/C, C/T] | 0.61 (0.46–0.79) | 0.0640 |
| rs1014971 [C/C] × rs11892031 [A/A] | 1.65 (1.26–2.15) | 0.0761 |
| rs9642880 [T/T] × rs710521 [A/A, A/G] | 1.75 (1.29–2.37) | 0.0087 |
| rs1014971 [C/T, T/T] × rs1495741 [A/A, A/G] | 0.62 (0.47–0.81) | 0.1051 |
| rs1014971 [C/C, C/T] × GSTM1 null | 1.73 (1.28–2.35) | 0.1111 |

The top ten of the 288 possible two-way interactions comprised of the six SNPs and GSTM1 as well as their odds ratios (OR) with 95% confidence intervals (CI) are listed in order of their p-values, where the p-values were adjusted for multiple comparisons by the Bonferroni correction.

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Information. Figures S2, S3 and S4 indicate that the variance of the test statistics determined in the 100,000 one degree of freedom, we examined the suitability of employing combinations. However, the approximation worked well for most of the combinations of two SNPs were larger than the ones for, for example, the number of SNPs forming an interaction increased. Surprisingly, the most extreme differences in p-values for the combinations of two SNPs were larger than the ones for, for example, three-way interactions. This, however, was only relevant for a few combinations.

Stability of the Estimated ORs

The above results, together with the relatively small case numbers in the subgroups of current, former and non-smoker for combinations of more than three SNPs, led us to focus on the interaction of two and three polymorphisms when we analyzed the stability of the ranks of the SNP combinations in the bootstrap samples (Tables S12, S13, S14, S15 and S16). In addition, the top two-way interactions occurred among the top ten interactions in a large majority of the bootstrap samples (Tables S12, S13, S14, S15 and S16). However, the instability of the ranks increased with the number of polymorphisms forming a combination (for example, the ranks for three-way SNP combinations in Tables S17, S18, S19, S20 and S21).

Differences in relevant SNP interactions between smokers and non-smokers. Interestingly, different SNP combinations were obtained for non-smokers and smokers. The optimal three-way SNP combinations (resulting in maximal odds ratios) for non-smokers consisted of (i) rs1014971, (ii) rs9642880, and (iii) one of the three SNPs: rs11892031, rs1495741, or rs710521 (Tables 2 and 4 as well as Table 5). In contrast, the optimal combinations for the current smokers were composed of GSTM1, rs1014971, and one of the three SNPs: rs11892031,

### Table 3. Top ten three-way interactions found in the analysis of the ever smokers.

| SNP combination | OR (95% CI) | P-value |
|-----------------|-------------|---------|
| rs8102137 [C/T, T/T] × rs11892031 [A/A] × GSTM1 null | 1.58 (1.30–1.92) | 0.0059 |
| rs710521 [A/A, A/G] × rs11892031 [A/A] × GSTM1 null | 1.51 (1.26–1.80) | 0.0080 |
| rs710521 [A/A, A/G] × rs11892031 [C/A] × GSTM1 null | 1.55 (1.28–1.88) | 0.0135 |
| rs9642880 [G/G, G/T] × rs710521 [A/A, A/G] × GSTM1 present | 0.66 (0.53–0.79) | 0.0171 |
| rs8102137 [C/T, T/T] × rs1495741 [A/A, A/G] × GSTM1 null | 1.52 (1.26–1.84) | 0.0248 |
| rs8102137 [C/T, T/T] × rs11892031 [A/A, A/C] × GSTM1 null | 1.50 (1.25–1.81) | 0.0315 |
| rs1014971 [C/C, C/T] × rs11892031 [A/A] × GSTM1 null | 1.46 (1.22–1.74) | 0.0419 |
| rs9642880 [G/G, G/T] × rs11892031 [A/A, A/C] × GSTM1 present | 0.68 (0.57–0.82) | 0.0520 |
| rs710521 [A/A, A/G] × rs11892031 [A/A, A/C] × GSTM1 null | 1.45 (1.22–1.72) | 0.0552 |
| rs710521 [A/A, A/G] × rs1014971 [C/C, C/T] × GSTM1 present | 0.69 (0.57–0.82) | 0.0582 |

The top ten of the 1,760 possible three-way interactions comprised of the six SNPs and GSTM1, as well as their odds ratios (OR) with 95% confidence intervals (CI) are listed in order of their p-values, where the p-values were adjusted for multiple comparisons by the Bonferroni correction.

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### Table 4. Top ten three-way interactions found in the analysis of the non-smokers.

| SNP combination | OR (95% CI) | P-value |
|-----------------|-------------|---------|
| rs9642880 [G/T, T/T] × rs710521 [A/A, A/G] × rs1014971 [C/C] | 1.98 (1.49–2.63) | 0.0044 |
| rs9642880 [G/T, T/T] × rs1014971 [C/C] × rs1014971 [A/A, A/G] | 1.95 (1.47–2.58) | 0.0054 |
| rs9642880 [G/T, T/T] × rs1014971 [C/C] × rs11892031 [A/A, A/C] | 1.93 (1.46–2.55) | 0.0061 |
| rs9642880 [G/T, T/T] × rs1014971 [C/C] × GSTM1 null | 2.21 (1.58–3.10) | 0.0070 |
| rs9642880 [G/G, G/T] × rs1014971 [C/T, T/T] × rs1014971 [C/C, C/T] | 0.54 (0.40–0.71) | 0.0318 |
| rs9642880 [G/G, G/T] × rs1014971 [C/T, T/T] × rs1014971 [A/A, A/G] | 0.54 (0.40–0.71) | 0.0325 |
| rs9642880 [G/G, G/T] × rs1014971 [C/T, T/T] × rs1495741 [A/A, A/G] | 0.56 (0.42–0.74) | 0.0735 |
| rs710521 [A/A, A/G] × rs1014971 [C/C] × GSTM1 null | 1.93 (1.41–2.64) | 0.0773 |
| rs9642880 [G/T, T/T] × rs1014971 [C/C] × rs11892031 [A/A] | 1.80 (1.35–2.40) | 0.0954 |
| rs710521 [A/A, A/G] × rs1014971 [C/C] × rs11892031 [A/A] | 1.74 (1.33–2.29) | 0.1142 |

The top ten of the 1,760 possible three-way interactions comprised of the six SNPs and GSTM1, as well as their odds ratios (OR) with 95% confidence intervals (CI) are listed in order of their p-values, where the p-values were adjusted for multiple comparisons by the Bonferroni correction.

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Comparison with Published Results

Most UBC cases can clearly be attributed to cigarette smoking (Table 8; present study PAR: 46%; other studies PAR: 50–56%). While current smokers have an approximately 3-fold risk (present study OR = 3.28, other studies OR = 2.77–4.95) of developing UBC – increasing with amount and time – the UBC risk of former smokers decreases to an OR of about two (present study OR = 2.12, other studies OR = 1.74–2.34). Both subgroups contribute almost equally to the UBC cases in the present study (former smokers PAR = 29%, current smokers PAR = 30%), whereas in published studies estimates of the PAR range from 28–40% for former smokers to 39% in current smokers. Interestingly, among men more UBC cases are attributable to smoking (former 41%, current 55%, ever 66%) than among women (former 17%, current 32%, ever 30%).

Table 6. Population attributable risks and odds ratios due to genetic factors.

| Genetic Factors | Present study | Published |
|-----------------|---------------|-----------|
|                 | PAR | OR      | PAR | OR      |
| All             | –   | –       | 30% [47,51] | 1.04–1.81 [1,9,31,48] |
| GSTM1           | 13%a | 1.28 | 14–26% [47,48,54,55] | 1.28–1.70 [47,48,54,55] |
| NAT2            | 1%b | 1.02b | 8.2% c [54] | 1.04–1.43 [47,48,54,55] |
| "wimp" SNPs     | 33%a | 1.02–1.34a | – | 1.11–1.81 [1,9,32] |
| Top 3-way interaction | 16% | 1.48 | – | – |

Population attributable risks (PARs) and odds ratios (ORs) were calculated from the data of the present study and summarized from previously published studies for different genetic factors. Numbers in brackets refer to the publications in which the PARs and ORs were published.

aAdjusted for age, gender, smoking habits, all measured SNPs and study site; crude PAR/OR: 16% / 1.39; adjusted for age and gender: 16 % / 1.37; adjusted for all measured SNPs: 15%/1.36.

bAdjusted for age, gender, smoking habits, all measured SNPs and study site; crude PAR/OR: 5% / 1.09; adjusted for age and gender: 3% / 1.05; adjusted for all measured SNPs: 5%/1.10.

*Data from Moore et al. [48] and Garcia-Closas et al. [47] result in PARs of 2–18%.

*Combined PAR, individual SNP OR and PAR adjusted for age, gender, smoking habits and all measured SNPs.

*Range of individual SNP OR adjusted for age, gender, smoking habits, all measured SNPs and study site depending on the mode of inheritance.

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Comparison of the Results from Analyzing Non-smokers and Smokers

The distinct SNP patterns for smokers and non-smokers found in our analysis are remarkable, since the genes closest to the top scoring “smoker variants” are involved in the detoxification of carcinogens in cigarette smoke, whereas the top scoring “non-smoker SNPs” are associated with cell cycle control and DNA stability. The deletion variant of \( \text{GSTM1} \), the polymorphism found in our analysis to be the most important in smokers, results in loss of activity of the phase II metabolizing enzyme glutathione S-transferase M1, which is involved in detoxification of numerous polycyclic aromatic hydrocarbons [39,40]. The second scoring “smoker variant” rs11892031 is located closest to the \( \text{UGT1A} \) cluster [9]. UDP-glucuronosyltransferase is also a phase II metabolizing enzyme responsible for the conjugation and detoxification of several urinary bladder carcinogens present in cigarette smoke [27,41–45].

In contrast, the two top scoring “non-smoker SNPs” are not involved in carcinogen detoxification. Rs1014971 is located approximately 25 kb centromeric of \( \text{APOBEC3A} \), which deaminates cytosine to uracil, thereby playing a role in endogenous mutagenesis [1,9]. The second scoring “smoker variant” rs11892031 is located closest to the \( \text{UGT1A} \) cluster [9]. UDP-glucuronosyltransferase is also a phase II metabolizing enzyme responsible for the conjugation and detoxification of several urinary bladder carcinogens present in cigarette smoke [27,41–45].

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Another striking observation is that the three SNPs forming the optimal three-way SNP combination in non-smokers, i.e. rs9642880[G/T, T/T] x rs710521[A/A, A/G] x rs1014971[C/G]...

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**Table 7.** Population attributable risks and odds ratios for different occupational exposures.

| Increased risk | Occupation/Exposure | Present study PAR | OR | Published PAR | OR |
|----------------|---------------------|-------------------|----|---------------|----|
| All            | –                   | 20–26% [35–37]    | –  | 7–10% [33,34] | –  |
| Moderately     | Painter             | 0.89%             | 1.38 | 0.7% [33,34] | 1.17–1.98 [36,38,57–59] |
|                | Hairdresser         | –                 | –   | 0.2% [33,34] | 1.23–2.10 [36,38,63] |
|                | Coal miner          | 2.81%             | 1.47 | –             | 1.31–2.40 [38,58,64,65] |
| Clearly        | Aluminium Worker    | –                 | –   | –             | 1.50–2.34 [36,66] |
|                | Rubber Industry     | 2.80%             | 1.76 | –             | 1.29–1.30 [36,38] |
|                | Roofer and Slater   | –                 | –   | –             | 1.70 [36] |
| Strongly       | Benzidine/\(-\)Naphthylamine | – | – | 1.60 [37] |
|                | Benzidine\(^{b}\)   | –                 | –   | –             | 30–75 [67,68] |
|                | \(-\)Naphthylamine\(^{c}\) | – | – | 5–200 [68] |
|                | 4-Aminobiphenyl\(^{d}\) | – | – | 11%\(^{d}\) [68] |
|                | 4-Chloro-o-toluidine | –                 | –   | –             | 38–90 [68] |

Population attributable risks (PARs) and odds ratios (ORs) were calculated from the data of the present study and summarized from previously published studies for different occupations and occupational exposures, partly stratified by gender (M: Male, F: Female). Numbers in brackets refer to the publications in which the PARs and ORs were published.

\(^{a}\)Painters before 1960 had a clearly increased risk: OR = 2.42–2.78 [60–62].
\(^{b}\)More exactly, Aluminium Workers (Soderberg Processing).
\(^{c}\)Results from historical studies.
\(^{d}\)Prevalence in exposed workers.

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**Table 8.** Population attributable risks and odds ratios in the different smoker groups.

| Smoking habits | Present study | Published |
|----------------|--------------|-----------|
|                | PAR | OR | PAR | OR |
| Former smokers | 30%\(^{a}\) | 2.12\(^{a}\) | 28–40% [48,53] | 1.74–2.34 [48,53,71] |
|                | M: 41% [69] | F: 17% [70] | M: 2.74 [69] | F: 1.42 [70] |
| Current smokers | 29%\(^{b}\) | 3.28\(^{b}\) | 39% [48,53] | 2.77–4.95 [48,53,71] |
|                | M: 55% [69] | F: 32% [70] | M: 4.72 [69] | F: 1.89 [70] |
| Ever smokers   | 46%\(^{c}\) | 2.47\(^{c}\) | 50–56% [48,52,53] | 2.61–2.89 [48,53] |
|                | M: 66% [69] | F: 30% [70] | M: 3.65 [69] | F: 1.69 [70] |

Population attributable risks (PARs) and odds ratios (ORs) were calculated from the data of the present study and summarized from previously published studies for the different smoker groups, partly stratified by gender (M: Male, F: Female), where non-smokers were used as a reference group having no additional risk. Numbers in brackets refer to the publications in which the PARs and ORs were published.

\(^{a}\)Adjusted for age and gender; crude PAR/OR: 39%/2.65; adjusted for age, gender, SNPs: 30%/2.15.
\(^{b}\)Adjusted for age and gender; crude PAR/OR: 29%/3.21; adjusted for age, gender, SNPs: 28%/3.17.
\(^{c}\)Adjusted for age and gender; crude PAR/OR: 51%/2.83; adjusted for age, gender, SNPs: 46%/2.47.

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[C], differ from the three polymorphisms composing the optimal three-way interaction in ever smokers, i.e. rs8102137[C/T, T/T] x rs11892031[A/A] x GSTM1 null. Moreover, the optimal three-SNP combination in non-smokers results in an OR of 1.98 (95% Cl: 1.49–2.63) that is significantly higher (p-value: 1.78x10^-24) than the OR of this combination in the ever smokers (OR: 1.03, 95% CI: 0.86–1.24). Conversely, the optimal three-SNP combination in ever smokers exhibits an OR of 1.58 (95% CI: 1.30–1.92), which is substantially, but not significantly (p-value: 0.143) higher than the OR of this three-SNP combination in non-smokers (OR: 1.21; 95% CI: 0.90–1.64). However, cigarette smoking is already associated with an OR of 3.28 (95% CI: 2.67–4.03) when current smokers are compared to non-smokers in our study population (Table 9). This high OR suggests that under conditions of continuous exposure to cigarette smoke carcinogens, the contribution of the “non-smoker SNPs” with their relatively small influence on cell cycle and DNA integrity control, is of minor relevance.

Comparison with Published Results

To study the consistency of this observation, we re-visited the data of the genome-wide association study on UBC of Rothman et al. [9] who validated rs9642880 and rs710521 in 3,532 UBC cases and 5,120 controls, and confirmed the impact of the GSTM1 deletion in 2,480 cases and 3,222 controls. Assuming a multiplicative model, they also obtained higher ORs for non-smokers compared to ever smokers for rs9642880 (1.24 for non-smokers versus 1.16 for smokers) and rs11892031 (1.49 versus 1.31). The higher OR for rs9642880 contradicts the study of Kiemeney et al. [7] who reported no association of rs9642880 with smoking habits. Also, the findings of a higher OR for rs11892031 in non-smokers is

| Smoking Habit (nCa/nCo) | Cases       | Controls  | OR (95% Cl) | OR adj (95% CI adj) |
|------------------------|-------------|-----------|-------------|---------------------|
| Non-smokers (321/752)  | 21%         | 44%       | (reference) | (reference)         |
| Former smokers (742/656) | 50%        | 38%       | 2.65 (2.24–3.31) | 2.12 (1.78–2.53)    |
| Current smokers (431/315) | 29%        | 18%       | 3.21 (2.64–3.90) | 3.28 (2.67–4.03)    |
| Ever smokers (1173/971) | 79%        | 56%       | 2.83 (2.42–3.31) | 2.47 (2.10–2.90)    |

For each of the smoker subgroups containing nCa cases and nCo controls, the odds ratios (OR) and their corresponding 95% confidence intervals (95% CI) were computed, both not adjusted and adjusted for age and gender. The latter odds ratios are abbreviated by OR adj.

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Figure 2. Relative risks and frequency of risk factors assuming a PAR of 30%. Relative risks are calculated depending on the frequency of the risk factor in the population assuming a population attributable risk (PAR) of 30%, corresponding to the supposed PAR of genetic risk factors for UBC. Given a PAR of 30%, the relative risk does not fall below 1.43 if the frequency of the risk factor is present in almost the entire population. doi:10.1371/journal.pone.0051880.g002
in contrast to Tang et al. [32] who found a higher risk in ever smokers (OR = 1.29) than in non-smokers (OR = 1.23) based on a subset of study groups from Rothman et al. (GWAS stage 1 [9]). However, no difference was found for rs710521 (1.15 vs. 1.14) in accordance with the discovery GWAS [7], and an opposite trend was shown for rs1014971 (1.11 vs. 1.16) and the MTH2 tagging SNP rs1495741 (1.00 vs. 1.18) in accordance with the assumed higher risk of slow acetylators in smokers. Therefore, the difference should still be interpreted with caution until independent confirmatory data are available.

The association among the GSTM1 null genotype, smoking habits and bladder cancer has been controversial since the first study by Bell et al. in 1993 [2]. In their study, smokers had an OR of 1.8 and non-smokers an OR of 1.3, indicating higher risks in smokers due to the lack of GSTM1. However, recent meta analyses and large or pooled studies found no or only weak evidence for an association between GSTM1 and smoking habits [9,46–48], whereas Rothman et al. [9] reported an even higher OR for non-smokers than for ever smokers (1.71 vs. 1.47). In this context, it should be mentioned that our study groups present a higher proportion of occupationally exposed bladder cancer cases. This may be particularly important for GSTM1. For example, it was shown that bladder cancer patients with occupational histories in coal, iron, and steel industries, i.e. exposure to polycyclic aromatic hydrocarbons, presented with high percentages of GSTM1 null genotypes [49]. Decades after the closure of these industries, the GSTM1 genotypes were equal in both cases and controls (GSTM1 null: 52%) [50].

Gain of Considering SNP Interactions

We have shown that SNP combinations result in less than additive ORs compared to the influence of the individual SNPs. For example, the ORs of the “non-smoker SNPs” rs1014971 and rs9642880 are 1.63 = 1/0.61 and 1.48, respectively, in non-smokers (for all ORs of individual SNPs, see Table S6). In comparison, the combination of both SNPs results in an OR of 1.91 in this subgroup (Table S16), which is larger than the individual effects, but smaller than 1.63+1.48 = 3.11. Adding a third SNP to the rs1014971 × rs9642880 combination results in an increase of only 0.07 (Table S21). The less than additive effect is not surprising considering the relatively high frequencies of the high-risk alleles (rs1014971 [C/C]: 40%; rs9642880 [G/T, T/T]: 71%; rs710521 [A/A, A/G]: 93%) in non-smoking controls and their overlap between individual SNPs (two-way interaction: 27%; three-way interaction 24%). Therefore, it seems unlikely that the addition of further “low impact” or “wimp SNPs” [1] would lead to a relevant increase in the combined ORs in populations of European descent.

Analysis of Population Attributable Risks

Altogether, it is estimated that up to 30% of bladder cancer cases can be explained by genetic risk factors [47,51] (see also Table 6), whereas about half of all UBC cases are caused by cigarette smoking [48,52,53] (see also Table 8). Estimates of the population attributable risk (PAR) for occupations vary widely, ranging from 7.1% in men and 1.9% in women [34] to 20–26% in both genders [35–37] (see also Table 7). The PAR – as a measure of the proportion of cases that could be explained by a certain risk factor – depends on and increases with both the frequency of the risk factor in the population and the relative risk (which is often approximated by the OR). Thus, assuming that the PAR of the genetic risk factors is limited to about 30% in the general population, the OR of the frequent combinations of these polymorphisms must be limited to modest ORs of about two (Figure 2). For instance, a PAR of 30% results from a risk factor present in 40% of the population and a relative risk of 2.1, whereas a risk factor present in 10% of the population requires a relative risk of 5.3 to obtain the same PAR. However, in subgroups different impacts of the genetic risk factors can be observed, not only in terms of relevance of single SNPs and their combinations, but also with respect to their combined attributable risks. In our study, combined PARs for the “wimp SNPs” range from 28% in ever smokers to 43% in non-smokers and also reflect the different impact of genetic risk factors in subpopulations with higher or lower exposure to bladder carcinogens from tobacco smoke.

Conclusion

In conclusion, we have shown that different types of genetic variants confer different susceptibility to smokers and non-smokers. In addition, the present work fuels the debate regarding the degree to which genetic disposition or environmental exposure contributes to carcinogenesis. Whereas the odds ratio of cigarette smoking is approximately 3.5 for current smokers in most studies, the combined high-risk alleles of the SNPs recently discovered in genome-wide association studies add up to ORs of approximately 2.0. Therefore, the environmental factors seem to have a higher impact on the UBC risk than genetic disposition based on the SNPs derived from recent genome-wide association studies.

Supporting Information

Figure S1 Test statistics for the optimal combinations consisting of one to seven SNPs in the bootstrap samples. Data are shown for (A) the total group, (B) the ever smokers, (C) the current smokers, (D) the former smokers, and (E) the non-smokers. For each of the optimal combinations from in Figure 1A, box plots of the test statistics in the 500 bootstrap samples drawn from the respective subgroups are displayed. The plots correspond to the odds ratios shown in Figure 1B-F. The crosses mark the test statistics for the respective optimal combinations.

Figure S2 Mean test statistic over 100,000 permutations of the case-control status. For the top 100 SNP combinations of each size and in each subgroup, the means over the Wald statistics in 100,000 permutations of the case-control status were computed. The subgroup-wise distributions of these means are shown as box plots, and the subgroup-wise means of the top 10 combinations are marked by red crosses. For a better representation, six outliers (with means smaller than 0.9) were removed from the box plots for the two-way interactions. For reference, the minimum and maximum of the sample means from 100 samples consisting of 100,000 random draws from a χ²-distribution with 1 degree of freedom are marked by dashed blue lines. If the χ²-approximation is reasonable, the mean test statistic over the 100,000 permutations should be approximately 1, i.e. close to the solid blue lines marking the mean of the χ²-distribution with 1 degree of freedom.

Figure S3 Variance of the test statistic over 100,000 permutations of the case-control status. In addition to the mean test statistic displayed in Figure S2, the variances of the test statistics for the top 100 interactions in the different subgroups were computed. The subgroup-wise distributions of these variances are shown as box plots, and the variances of the top ten combinations in the subgroups are marked by red crosses. For a better representation, six outliers (with variances smaller than...
1.3) were removed from the box plots for the two-way interactions. For reference, the minimum and maximum of the sample variances from 100 samples consisting of 100,000 random draws from a \( \chi^2 \)-distribution with 1 degree of freedom are marked by dashed blue lines. If the \( \chi^2 \)-approximation is reasonable, the variance of the test statistic over the 100,000 permutations should be approximately 2, i.e., close to the solid blue lines marking the variance of the \( \chi^2 \)-distribution with 1 degree of freedom.

Figure S4 Differences between parametric and permutation-based p-values. Box plots of the differences between the parametric p-values of the top 100 SNP combinations of each size from the analysis of each subgroup and the corresponding p-values computed based on 100,000 permutations of the case-control status. The differences of the respective top ten SNPs are additionally marked by red crosses. Ideally, this difference is zero (which is marked by a dashed blue line). The six outliers removed from Figures S2 and S3 (with means smaller than 0.9 and variances smaller than 1.3) were also removed before constructing the box plots.

Table S1 Distribution of gender in the study groups.
Table S2 Distribution of age at diagnosis (cases) or examination (controls) in the study groups.
Table S3 Frequency of non-smokers, former smokers and current smokers in the study groups.
Table S4 Chromosomal and data base information on the six analyzed SNPs.
Table S5 Testing for Hardy-Weinberg equilibrium.
Table S6 Maximum odds ratios of the seven polymorphisms in the subgroups.
Table S7 Stability of the ranks of the top ten individual effects in the total study group.
Table S8 Stability of the ranks of the top ten individual effects in the ever smoker group.
Table S9 Stability of the ranks of the top ten individual effects in the current smoker group.
Table S10 Stability of the ranks of the top ten individual effects in the former smoker group.
Table S11 Stability of the ranks of the top ten individual effects in the non-smoker group.
Table S12 Stability of the ranks of the top ten two-way interactions in the total study group.
Table S13 Stability of the ranks of the top ten two-way interactions in the ever smoker group.
Table S14 Stability of the ranks of the top ten two-way interactions in the current smoker group.
Table S15 Stability of the ranks of the top ten two-way interactions in the former smoker group.
Table S16 Stability of the ranks of the top ten two-way interactions in the non-smoker group.
Table S17 Stability of the ranks of the top ten three-way interactions in the total study group.
Table S18 Stability of the ranks of the top ten three-way interactions in the ever smoker group.
Table S19 Stability of the ranks of the top ten three-way interactions in the current smoker group.
Table S20 Stability of the ranks of the top ten three-way interactions in the former smoker group.
Table S21 Stability of the ranks of the top ten three-way interactions in the non-smoker group.
Appendix S1 Details on the polymorphisms.
Appendix S2 Details on the statistical analysis.

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Conceived and designed the experiments: JGH KG HS SS KI RM. Performed the experiments: MB. Analyzed the data: HS SS. Wrote the paper: JGH KG HS SS RM MB KI.
References

1. Golka K, Selinski S, Lehmann ML, Blaszczewski M, Marchan R, et al. (2011) Genetic variants in urinary bladder cancer: collective power of the “wimp SNP” panel. Nat Genet 43: 539–544.

2. Bell DA, Taylor JA, Paulson DF, Robertson CN, Mohler JL, Lucier GW (1993) Genetic risk and carcinogen exposure: a common inherited defect of the carcinoogen-metabolism gene glutathione S-transferase M1 (GSTM1) that increases susceptibility to bladder cancer. J Natl Cancer Inst 85: 1159–1164.

3. Cartwright RA, Glushan RW, Rogers HJ, Ahmad RA, Barham-Hall D, et al. (1984) Role of N-acetyltransferase phenotypes in bladder carcinogenesis: a pharmacogenetic epidemiological approach to bladder cancer. Lancet 2: 842–845.

4. Golka K, Prior V, Blaszczewski M, Casarović I, Schips W, et al. (1996) Occupational history and genetic N-acetyltransferase polymorphism in urotheelial cancer patients of Leverkusen, Germany. Scand J Work Environ Health 22: 332–338.

5. Kempleis M, Golka K, Reich S, Reckworth T, Bolt HM (1996) Glutathione S-transferase GSTM1 null genotypes as potential risk factors for urothelial cancer of the bladder. Arch Toxicol 71: 123–126.

6. Hengelder JG, Arand M, Herrero ME, Oesch F (1998) Polymorphisms of N-acetyltransferases, glutathione S-transferases, microsomal epoxide hydrolase and sulfotransferases: influence on cancer susceptibility. Recent Results Cancer Res 154: 47–85.

7. Kiemeyer LA, Thorlacius S, Sulem P, Geller F, Aben KK, et al. (2008) Sequence variant on 4q24 confers susceptibility to urinary bladder cancer. Nat Genet 40: 1307–1312.

8. Kiemeyer LA, Sulem P, Beesonbacher S, Vermeulen SH, Sigurdsson A, et al. (2009) A sequence variant at rs451633 confers susceptibility to urinary bladder cancer. Nat Genet 41: 415–419.

9. Rothman N, Garcia-Closas M, Chatterjee N, Malats N, Wu X, et al. (2010) A multi-stage genome-wide association study of bladder cancer identifies multiple susceptibility loci. Nat Genet 42: 970–974.

10. Rafnar T, Sulem P, Stacey SN, Geller F, Gudmundsson J, et al. (2009) Sequence variants at the TERT-CLPTM1L locus associate with many cancer types. Nat Genet 41: 221–227.

11. Rafnar T, Vermeulen SH, Sulem P, Thorleifsson G, Aben KK, et al. (2011) European genome-wide association study identifies SLC14A1 as a new urinary bladder cancer susceptibility gene. Hum Mol Genet 20: 4288–4291.

12. Wu X, Ye Y, Kiemeyer LA, Sulem P, Rafnar T, et al. (2009) Genetic variation in the prostate stem cell antigen gene PCSA confers susceptibility to urinary bladder cancer. Nat Genet 41, 991–995. Erratum in: Nat Genet 41: 1156.

13. Golka K, Hermes S, Selinski S, Blaszczewski M, Bolt HM, et al. (2009) Susceptibility to urinary bladder cancer: relevance of rs8646280[T], GSTM1 0/0 and occupational exposure. Pharmacogenet Genomics 19: 903–906.

14. Selinski S, Lehmann ML, Blaszczewski M, Ovsiannikov D, Moormann O, et al. (2012) Rs710521[A] on chromosome 3q28 close to TP63 is associated with increased urinary bladder cancer risk. Arch Toxicol 86: 647–658.

15. Krause G, Muller M, Lewalter J, Schulte PA (2006) The genetic and environmental factors involved in benzidine metabolism and bladder carcinoma in occupational exposure. Scand J Urol Nephrol 40 (Suppl 218): 58–63.

16. Silverman DT, Levin LI, Hoerer RN, Hartge P (1989) Occupational risks of bladder cancer in the United States today. J Natl Cancer Inst 66: 1191–1308.

17. Rusthon L, Bagga S, Bevan R, Brown TP, Cherrue JW, et al. (2010) Occupation and cancer in Britain. Br J Cancer 102: 1420–1437.

18. Doll R, Pete R (1981) The causes of cancer: Quantitative estimates of avoidable risks of cancer in the United States today. J Natl Cancer Inst 66: 1191–1308.

19. Karcev T, LeMasters GK, Ruder AM, Schulte PA (2006) The genetic and environmental factors involved in benzidine metabolism and bladder carcinoma in occupational exposure. Scand J Urol Nephrol 40 (Suppl 218): 64–78.

20. Ketterer B, Harris JM, Talakaga M, Meyer DJ, Pembble SE, et al. (1992) The human glutathione S-transferase supergene family, its polymorphism, and its effects on susceptibility to bladder cancer. Environ Health Perspect 90: 87–94.

21. Lee KH, Lee J, Ha M, Choi JW, Cho SH, et al. (2002) Influence of polymorphism of GSTM1 gene on association between glycolipidin a mutant frequency and urinary PAH metabolites in incinerator workers. J Toxicol Environ Health A 65: 1553–1567.

22. Inman CL, Iwamoto H, Chen WW, Yip PS, Ahuja N, et al. (2012) Rs507000[A] on chromosome 7q32 close to TP53 is associated with increased urinary bladder cancer risk. Arch Toxicol 86: 967–970.

23. Lehmann ML, Selinski S, Blaszczewski M, Orlich M, Ovsiannikov D, et al. (2010) Rs710521[A] on chromosome 3q28 close to TP63 is associated with increased urinary bladder cancer risk. Arch Toxicol 84: 967–970.

24. Selinski S, Lehmann ML, Blaszczewski M, Ovsiannikov D, Moormann O, et al. (2012) Rs11892031[A] on chromosome 2q37 in an intron region of the UGT1A locus is associated with urinary bladder cancer risk. Arch Toxicol 86: 1369–1378.

25. Federico A, Pallante P, Bianco M, Ferraro A, Esposito F, et al. (2009) Chromobox protein homologue 7 protein, with decreased expression in human bladder cancer, regulates cell cycle progression genes. Cancer Res 69: 7079–7087.

26. Vaerman J-P, Osrin D, Henry M, Gobin-Hobson S (2008) Evidence for editing of human papillomavirus DNA by APOBEC3 in benign and precancerous lesions. Science 320: 230–233.

27. Hengelder JG, Utesch D, Steinberg P, Platt KL, Diener B, et al. (2000) Cryopreserved primary hepatocytes as a constantly available in vitro model for the evaluation of human and animal drug metabolism and enzyme induction. Drug Metab Rev 32: 81–118.

28. Straubrh G, Lankisch TO, Manns MP, Elmer U (2008) Family 1 uridine-5-diphosphate glucuronosyltransferases (UGT): from Gilbert’s syndrome to genetic organization and variability. Arch Toxicol 82: 415–433.

29. Garcia-Closas M, Hein DW, Silverman D, Malats N, Yeager M, et al. (2011) SNP meta-analysis and meta-analysis of glutathione S-transferase M1 and bladder cancer risk. Carcinogenesis 32: 47–85.

30. Koff A, Cross F, Fisher A, Schumacher, J, Leggett K, et al. (1991) Human cyclin E, a new cyclin that interacts with two members of the CDC2 gene family. Cell 66: 1217–1220.
cohorts of twins from Sweden, Denmark, and Finland. N Engl J Med 343: 78–85.

52. Yu MC, Skipper PL, Tannenbaum SR, Chan KK, Ross RK (2002) Arylamine exposures and bladder cancer risk. Mutat Res 506–507: 21–28.

53. Puente D, Hartge P, Greiser E, Cantor KP, King WD, et al. (2006) A pooled analysis of bladder cancer case-control studies evaluating smoking in men and women. Cancer Causes Control 17: 71–79.

54. Vines P (2002) The relationship between polymorphisms of xenobiotic metabolizing enzymes and susceptibility to cancer. Toxicology 181–182: 457–462.

55. Zhang R, Xu G, Chen W, Zhang W (2011) Genetic polymorphisms of glutathione S-transferase M1 and bladder cancer risk: a meta-analysis of 26 studies. Mol Biol Rep 38: 2491–2497.

56. Vines P, Marinelli D, Autrup H, Brockmoller J, Casorbi I, et al. (2001) Current smoking, occupation, N-acetyltransferase-2 and bladder cancer: a pooled analysis of genotype-based studies. Cancer Epidemiol Biomarkers Prev 10: 1249–1252.

57. Bolm-Audorff U, Jockel KH, Kalgus B, Pohlabern H, Siepenkothen T (1993). Bösartige Tumoren der ableitenden Harnwege und Risiken am Arbeitsplatz. Scientific report Fb 697 from the report series of the Federal Institute for Occupational Safety and Health, Dortmund, Germany. Bremerhaven, Germany: Wissenschaftsverlag NW. In German.

58. Golka K, Bandel T, Reckwitz T, Urfer W, Bolt HM, et al. (1999) Occupational risk factors for bladder carcinoma. A case control study. Urologe A 38: 358–363. In German.

59. Guha N, Steenland NK, Merletti F, Altiere A, Cogliano V, Straif K (2010) Bladder cancer risk in painters: a meta-analysis. Occup Environ Med 67: 568–573.

60. Golka K, Bandel T, Schlafke S, Reich SE, Reckwitz T, et al. (1996) Urothelial cancer of the bladder in an area of former coal, iron, and steel industries in Germany: a case-control study. Int J Occup Environ Health 4: 79–84.

61. Golka K, Goebell PJ, Rettenmeier AW (2007) Bladder cancer: etiology and prevention. Dtsch Arztebl 104: A 719–723.

62. Myslak ZW, Bolt HM, Brockmann W (1991) Tumors of the urinary bladder in painters: a case-control study. Am J Ind Med 19: 705–713.

63. Colk JS, Baris D, Stewart P, Schned AR, Heaney JA, et al. (2004) Occupation and bladder cancer risk in a population-based case-control study in New Hampshire. Cancer Causes Control 15: 759–769.

64. Cordier S, Clavel J, Limasset JC, Boccou-Gibod L, Le Moual N, et al. (1993) Occupational risks of bladder cancer in France: a multicentre case-control study. Int J Epidemiol 22: 403–411.

65. Schillers E, Jamart J, Renard V (1987) Tobacco and occupation as risk factors in bladder cancer: a case-control study in southern Belgium. Int J Cancer 39: 287–292.

66. The´riault G, Tremblay C, Cordier S, Gingras S (1984) Bladder cancer in the aluminium industry. Lancet 1: 947–950.

67. Golka K, Prior V, Blaszewicz M, Bolt HM (2002) The enhanced bladder cancer susceptibility of NAT2 slow acetylators towards aromatic amines: a review considering ethnic differences. Toxicol Lett 128: 229–241.

68. Golka K, Wiese A, Assenmato G, Bolt HM (2004) Occupational exposure and urological cancer. World J Urol 21: 382–391.

69. Brennan P, Bogillot O, Cordier S, Greiser E, Schill W, et al. (2000) Cigarette smoking and bladder cancer in men: a pooled analysis of 11 case-control studies. Int J Cancer 86: 289–294.

70. Brennan P, Bogillot O, Greiser E, Chang-Claude J, Wahrendorf J, et al. (2001) The contribution of cigarette smoking to bladder cancer in women (pooled European data). Cancer Causes Control 12: 411–417.

71. Gandini S, Botteri E, Iodice S, Bonati M, Lowenthal AB, et al. (2008) Tobacco smoking and cancer: a meta-analysis. Int J Cancer 122: 155–164.