The influence of glutathione S-transferases M1 and M3 on the development of bladder cancer

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Abstract

Problem: Cigarette smoking is the most important risk factor of transitional cell carcinoma of the urinary bladder. The effect of the glutathione S-transferases M1 (*GSTM1*) and M3 (*GSTM3*) on the influence of this risk factor was investigated.

Methods: A total of 293 bladder cancer patients from Dortmund and Wittenberg as well as 176 surgical patients without any malignancy from Dortmund were genotyped for *GSTM1* und *GSTM3* according to standard PCR/RFLP methods. Smoking habits were also qualified by a standardized interview.

Results: The percentage of *GSTM1* negative cases was 63 % in the entire bladder cancer patient group compared to 50 % in the control group. *GSTM3*A/*A* genotype was 76 % in the entire group of bladder cancer cases and 74 % in controls. Smokers and ex-smokers were overrepresented in the bladder cancer patient group. A significant association between smoking status and *GSTM*1 or *GSTM3* genotype could not be revealed.

Conclusion: The elevated percentage of *GSTM1* negative bladder cancer cases shows the important effect of this polymorphic enzyme on the development of bladder cancer. In contrast to some other studies, an influence of *GSTM1* on the risk due to cigarette smoking could not be observed.

Key Words: Bladder cancer, glutathione S-transferase M1, glutathione S-transferase M3, smoking

1 Introduction

In 1998, a total of 10,546 bladder cancer cases in Germany among men and 5,190 cases among women were newly diagnosed (ARBEITSGEMEINSCHAFT BEVÖLKERUNGSBEZOGENER KREBSREGISTER IN DEUTSCHLAND, 2002).

The transitional cell carcinoma (TCC; synonym: urothelial cancer) of the urinary bladder is a typical example of chemical induced carcinogenesis. For example, aromatic amines and soluble azo dyes which can be released in the human organism could be revealed as carcinogens in many former studies (MYSLAK UND BOLT, 1988; VINEIS, 1994; GOLKA ET AL., 2004).

In 1993, BELL ET AL. showed that the majority of patients suffering from transitional cell carcinoma of the bladder who smoked a certain amount of cigarettes were lacking the gene for glutathione S-transferase M1 (GSTM1). A significantly higher incidence of the homozygous deletion of the GSTM1 genotype (0/0) was also found in bladder cancer patients from Dortmund, a former area of former coal, iron and steel industries (KEMPKES ET AL., 1996).

This study was conducted to investigate a connection between the smoking status and the genotype of two polymorphic glutathione S-transferases M1 and M3 in bladder cancer patients from industrial areas and to send the results to the International Collaborative Study on Genetic Susceptibility to Environmental Carcinogens (GSEC; principal investigators: Prof. Vineis, Turino; Dr. Taioli, Milano) for further analyses in a pooled study.

1.1 The superfamiliy of glutathione S-transferases

Whereas cytochrome P450 monooxygenases are enzymes responsible for the primary oxidation of xenobiotics (“phase I metabolism”), glutathione S-transferases (GSTs) are a superfamily of enzymes involved in conjugation (“phase II metabolism”). GSTs seem to have two major functions. First of all, they facilitate the conjugation of phase I products with the endogenous tripeptide glutathione, which acts as an essential cofactor of glutathione S-transferase activity (FJELLSTEDT ET AL., 1973; HABIG ET AL., 1974; MANNERVIK AND DANIELSON, 1988).

Another function of the GSTs is the non-catalytic intracellular transport of non-polar molecules (e.g., heme, bilirubin, bile acids) in the liver (ISHIGAKI ET AL., 1989).

Conjugation reactions facilitate the detoxification of electrophilic substances (CHASSEAUD, 1979). Electrophilic substances react with the nucleophilic SH-group of the glutathione-cysteinyl residue, resulting in covalent binding to the glutathione molecule. In the next metabolic step, the GSH-conjugates are cleaved by gamma-glutamyl-transpeptidases and dipeptidases, followed by acetylation to facilitate renal excretion.

Important carcinogenic substrates of GSTs include polycyclic aromatic hydrocarbons (pah; e.g., benz(a)pyrene (ROBERTSON ET AL., 1986), aflatoxin B1 (RANEY ET AL., 1992) or styrene oxide (PACIFICI ET AL., 1987).

1.1.2 Genetic polymorphisms of GSTM1 and GSTM3

The genes coding for GST enzymes are arranged in clusters in the human genome, with each cluster containing several highly homologous genes. The class μ gene cluster is found at gene locus 1p13 and consists of 5 genes (CANTLAY ET AL., 1994). Most authors concluded that about 50% of the Caucasian population lack the gene of glutathione S-transferase M1 (e.g., BROCKMÖLLER ET AL., 1994).

In addition, a further polymorphic site has been described in the *GSTM1* gene – a single C→G nucleotide substitution in exon 7 leading to an amino acid change, resulting in the existence of two variants of the active allele, *GSTM1*A* and *GSTM1*B*. Although these enzymes have different isoelectric points (6.1, 5.8, and 5.5, respectively), their specific activities towards typical substrates seem to be only marginally different (HAYES ET AL., 1989; WIDERSTEN ET AL., 1991).

In the *GSTM3* gene, a small deletion of 3 bp in intron 6, detected by a PCR-RFLP assay with *Mnl*I, has been reported (INSKIP ET AL., 1995). Although not within the encoding sequence, it appears to influence enzyme expression by creating a recognition motif for the versatile transcription factor YY1 in the *GSTM3*B* allele (INSKIP ET AL., 1995; YENGI ET AL., 1996). The variant allele *GSTM3*B* additionally appears to be in linkage disequilibrium with *GSTM1*A* (INSKIP ET AL., 1995). The presence of this *GSTM3* allele has been associated with both increased risk for certain types of cancer (SCHNAKENBERG ET AL., 2000; LOKTIONOV ET AL., 2001), as well as a protective effect for others (YENGI ET AL., 1996).

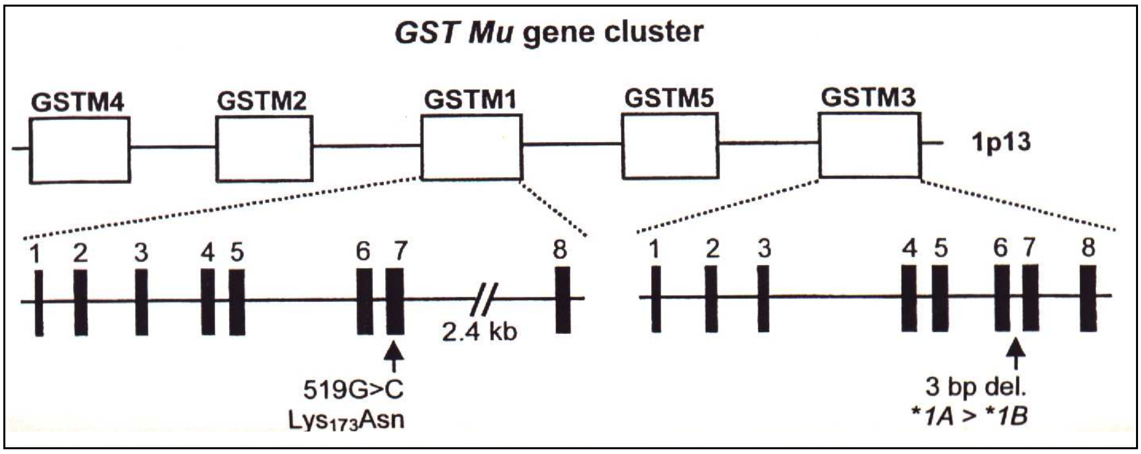

Figure 1: Glutathione S-transferase μ gene cluster with the alleles of the polymorphic *GSTM1* und *GSTM3* genes (from BROCKMÖLLER ET AL., 2001)

1.2 Transitional cell carcinoma

Most studies have shown an association between the *GSTM1* negative genotype and an increased risk of bladder cancer (ENGEL ET AL., 2002).

In contrast to studies concerning the *GSTM1* genotype, only a few studies are available which investigate an impact of the *GSTM3* genotype on the development of certain types of cancer.

In Germany, SCHNAKENBERG ET AL. (2000) investigated 146 patients suffering from bladder cancer as well as 206 healthy controls. The *GSTM3*A/*A* genotype was found in 171 of 206 controls (83.0 %), but only in 99 of 146 bladder cancer patients (67.8 %). The mutation of the *GSTM3* gene in intron 6 has been reported to be associated with a significantly elevated bladder cancer risk (OR 2.31, 95% CI 1.79-2.82).

1.2.1 Smoking and bladder cancer

The by far most important non-occupational risk factor for bladder cancer is smoking. It has been estimated (IARC, 2004) that in some areas up to 50 % of bladder cancer cases in men and up to 25 % in women are due to tobacco smoking. For men who are smoking cigarettes the bladder cancer risk is highest in Northern Italy and Spain and lowest in several eastern and northern European countries (NEGRI AND LA VECCHIA, 2001). A possible explanation is the different treatment of raw tobacco in different countries. CLAVEL ET AL. (1989) have reported that “black”, i.e. air-cured tobacco, is a stronger risk factor for bladder cancer than “blond“, i.e. flue-cured tobacco. In South East Europe, “black” tobacco is commonly preferred by smokers.

To date, some 4,000 compounds in tobacco smoke, thereof 69 carcinogens, have been identified (IARC, 2004). Very important in regard to the causes of bladder cancer in humans are polycyclic aromatic hydrocarbons, different aromatic amines which are proven carcinogens in humans like β-naphthylamine and 4-aminodiphenyl as well as nitrosamines. The concentrations of each of these compounds in tobacco smoke is, amongst others, dependent on the type of tobacco used, drying and processing.

1.2.2 Smoking, *GSTM1* genotype and bladder cancer

Glutathione S-transferases are important xenobiotic metabolizing enzymes which are involved in detoxification and elimination of important xenobiotics like polycyclic aromatic hydrocarbons (pah) or aromatic amines. These substances are also contained in tobacco smoke. The question raises whether these substances might be a risk factor for bladder cancer only for those who have a *GSTM*1 negative genotype and a smoking history. In other words, the question raises if the *GSTM1* negative genotype is generally a risk factor for bladder cancer or a risk factor only for those subjects with a very special history of exposure to particular combustion products from occupational and/or non-occupational sources.

2 Materials und Methods

2.1 Investigated subjects

In the present study, patients from two different urological departments were investigated in the time period from 1994 to 2000. Altogether, 293 bladder cancer patients and 176 patients without any known malignancy were investigated.

The bladder cancer patients were from the department of urology of the Klinikum Dortmund (transitional cell carcinoma patients (TCC Ca Do) n=83) and from the department of urology of the Paul-Gerhardt-Stiftung, Lutherstadt Wittenberg (transitional cell carcinoma patients Wittenberg (TCC Ca Witt) n=210). The patients of the control group were from the department of surgery of the Klinikum Dormund (control patients Dortmund (Controls Surg Do) n=176).

Demographic data (age, weight, gender, date of birth) and data from the medical history, especially those possibly relevant as risk factors for bladder cancer like occupational exposure to bladder carcinogenic substances and information on the smoking habits were collected (see dissertation Schmidt).

In all three groups, the portions of the *GSTM1* and *GSTM3* genotypes were investigated in the subgroups of smokers, ex-smokers and non-smokers. A person who reported of smoking a certain amount of cigarettes per day at the time of completing the questionnaire was defined as a smoker. Persons reporting not to smoke any more were classified as ex-smokers. Non smokers were persons who reported to have never smoked.

2.2 Statistic analysis

Differences between subgroups were checked using the *p*-values of the respective χ^2 test of homogeneity. Furthermore odds ratios and confidence limits were calculated. Note, that OR_{total} and CI_{total} refer to the odds ratio and the confidence interval of the total case group (cases of Dortmund and Wittenberg considered jointly), OR_{Do} and CI_{Do} refer to the odds ratio and the confidence interval of the Dortmund cases and controls and OR_{Witt} and CI_{Witt} refer to the odds ratio and the confidence interval of the Wittenberg cases with the cases of Dortmund as controls. The latter results have to be considered with caution due to potential differences between these two groups.

3 Results

3.1 Description and comparison of the investigated groups

In this study, 293 patients with a histologically ascertained transitional cell carcinoma of the urinary bladder as well as 176 surgical patients without any known malignancy have been investigated.

The majority of cases of the bladder cancer patients from Dortmund (51 %) as well as from Wittenberg (53 %) were older than 65 years. The observed differences were not significant ($\chi^2 = 8.3577$, $p = 0.21$).

Table 1: Age distributions in the investigated groups from Dortmund (controls) and Wittenberg und Dortmund (bladder cancer patients)

Age (years)	**< 58**	**59 – 65**	**66 – 72**	**> 72**	**Total**
Group					
Controls Surg Do	57 (32%)	47 (27%)	36 (21%)	36 (21%)	176
TCC Ca Witt	50 (24%)	50 (24%)	56 (27%)	54 (26%)	210
TCC Ca Do	18 (22%)	23 (28%)	17 (21%)	25 (30%)	83
TTC Ca Witt + TCC Ca Do	68 (23%)	73 (25%)	73 (25%)	79 (27%)	293
Total	125 (27%)	120 (27%)	109 (23%)	115 (25%)	469

Gender

Regarding the gender, noticeable differences could be observed between the investigated groups ($\chi^2 = 53.2392$, $p < 0.0001$; $OR_{total} = 4.9$, $CI_{total} = [3.1 – 7.7]$). Whilst in both bladder cancer groups males were clearly overrepresented (Wittenberg: 87 %, Dortmund: 86 %), there was only a slightly elevated portion of males in the control group (56 %).

Table 2: Gender in the investigated groups

Gender	**Male**	**Female**	**Total**
Group			
Controls Surg Do	99 (56%)	77 (44%)	176
TCC Ca Witt	182 (87%)	28 (13%)	210
TCC Ca Do	71 (86%)	12 (15%)	83
TCC Ca Witt + TCC Ca Do	253 (86%)	40 (14%)	293

Smoking habits

All patients of the three investigated groups were stratified for “smokers“, “ex-smokers“ and “non-smokers” according to the information taken from the questionnaires. Additionally, different parameters characterizing smoking habits like the number of pack years, age at the beginning of smoking and duration of non-smoking in ex-smokers were investigated.

The smoking habits in the two bladder cancer groups were quite different from that in the controls ($\chi^2 = 20.2769$, p = 0.0004). Both, smokers ($OR_{total} = 2.7$, $CI_{total} = [1.5 – 4.0]$; $OR_{Witt} = 2.1$, $CI_{Witt} = [1.2 – 3.6]$; $OR_{Do} = 3.7$, $CI_{Do} = [1.7 – 7.6]$) and ex-smokers ($OR_{total} = 2.3$, $CI_{total} = [1.5 – 3.7]$, $OR_{Witt} = 2.6$, $CI_{Witt} = [1.3 – 5.4]$; $OR_{Do} = 2.2$, $CI_{Do} = [1.4 – 3.7]$) showed an elevated risk of bladder cancer.

In bladder cancer patients from Wittenberg, compared to the bladder cancer patients from Dortmund, the portion of ex-smokers was slightly elevated. The portion of smokers in bladder cancer patients from Dortmund was higher than that from Wittenberg. In contrast, in the control group a large portion of non-smokers was observed (39 %). Five patients did not provide data on their smoking habits.

Table 3: Bladder cancer patients and controls classified for smoking status

Smoking status	**Ex-smoker**	**Smoker**	**Non-smoker**	**Total**
Group				
Controls Surg Do	59 (35%)	46 (27%)	66 (39%)	171
TCC Ca Witt	94 (45%)	69 (33%)	47 (22%)	210
TCC Ca Do	33 (40%)	36 (43%)	14 (17%)	83
TCC Ca Witt + TCC Ca Do	127 (43%)	105 (36%)	61 (21%)	283

Regarding the pack years, significant differences could be observed between the investigated groups ($\chi^2 = 65.4243$, $p < 0.001$). The opposed distribution in the two investigated bladder cancer groups is striking. A total of 26 % of the bladder cancer patients from Wittenberg reported 0-10 pack years. With increasing numbers of pack years, the percentage was decreasing almost arithmetically. Only 13 % reported on more than 40 pack years. An opposed trend was observed in bladder cancer patients from Dortmund: 2 % reported on 0-10 pack years, whereas 49 % reported on more than 40 pack years.

Table 4: Number of pack years reported in the investigated groups

Pack years	**0-10**	**11-20**	**21-30**	**31-40**	**> 40**	**Total**
Group						
Controls Surg Do	8 (8%)	7 (7%)	21 (20%)	39 (38%)	29 (28%)	104
TCC Ca Witt	40 (26%)	35 (23%)	32 (21%)	26 (17%)	20 (13%)	153
TCC Ca Do	1 (2%)	7 (13%)	9 (17%)	10 (19%)	26 (49%)	53
TCC Ca Witt + TCC Ca Do	41 (20%)	42 /21%)	41 (20%)	36 (18%)	46 (23%)	203

Patients from all three groups who reported to have smoked were also investigated for age at beginning of smoking. In all the three groups, a low age at starting the smoking career was observed.

The age at the beginning of smoking was quite different in the investigated groups ($\chi^2 = 9.7424$, $p = 0.0077$). Only 13 % of the control group were older than 25 years, in contrast to 33 % of the bladder cancer patients from Wittenberg as well as to 24 % of the bladder cancer patients from Dortmund. Thus, beginning of smoking at an age of < 20 could not be confirmed as a risk factor for bladder cancer (OR_{total} = 0.3, CI_{total} = [0.2 – 0.7])

Table 5: Age at the beginning of smoking

Age at beginning of smoking	**< 20 years old**	**> 25 years old**
Group		
Controls Surg Do	66 (87%)	10 (13%)
TCC Ca Witt	79 (67%)	39 (33%)
TCC Ca Do	31 (76%)	10 (24%)
TCC Ca Witt + TCC Ca Do	110 (69%)	49 (31%)

In another step, the subgroups of ex-smokers were investigated for possible differences in the duration of having quitted smoking until the time of interview. No significant differences were observed between the investigated groups (χ^2 = 7.2949, p = 0.29). Only 22 % of the bladder cancer patients from Wittenberg and 39 % of the bladder cancer patients from Dortmund reported a period of having quitted smoking ≤ 9 years, the remaining ex-smokers have quitted smoking for more than 9 years.

Table 6: Duration of abstinence in years from smoking in ex-smokers in the investigated groups

Abstinence (years)	**0-4**	**5-9**	**10-19**	**< 20**	**Total**
Group					
Controls Surg Do	10 (17%)	11 (19%)	15 (25%)	23 (39%)	59
TCC Ca Witt	11 (12%)	9 (10%)	30 (32%)	44 (47)	94
TCC Ca Do	7 (25%)	4 (14%)	9 (32%)	8 (29%)	28
TCC Ca Witt + TCC Ca Do	18 (15%)	13 (11%)	39 (32%)	52 (43%)	122

Tumor staging und grading

The extensions of the tumours in patients from Dortmund and Wittenberg were remarkably different ($\chi^2 = 38.5833$, $p < 0.0001$). In bladder cancer patients from Wittenberg, tumours staged Ta und T1 (i.e., low invasiveness) were more often seen than in patients from Dortmund (76 % *vs.* 66 %). Furthermore, no patient from Wittenberg was staged with T4 (i.e., high invasiveness), in contrast to 5 % of the patients from Dortmund.

Table 7: Tumour staging of bladder cancer patients from Dortmund and Wittenberg

Staging	**Ta**	**T1**	**T2**	**T3**	**T4**	**Total**
Group						
TCC Ca Witt	74 (36%)	82 (40%)	9 (4%)	42 (20%)	0	207
TCC Ca Do	20 (27%)	29 (39%)	17 (23%)	5 (7%)	4 (5%)	75
TCC Ca Witt + TCC Ca Do	94 (33%)	111 (39%)	26 (9%)	47 (17%)	4 (1%)	282

The results regarding the grading of the bladder cancer tumours from Dortmund and Wittenberg were close to significance ($\chi^2 = 6.6159$, $p = 0.085$). In more than 50 %, bladder cancer cases from Dortmund were graded G2; there was a trend for higher gradings in patients from Wittenberg.

Table 8: Grading of bladder cancer patients from Dortmund and Wittenberg

Grading	**G1**	**G2**	**G3**	**G4**	**Total**
Group					
TCC Ca Witt	56 (27%)	76 (37%)	74 (36%)	1 (1%)	207
TCC Ca Do	21 (28%)	38 (51%)	16 (21%)	0	75
TCC Ca Witt + TCC Ca Do	77 (22%)	114 (40%)	90 (32%)	1 (1%)	282

3.2 Glutathione S-transferase M1 genotyping

Distribution of the *A and *B alleles in the investigated groups

No statistically significant differences in the distribution of the investigated *GSTM1* negative genotype was observed in the bladder cancer groups from Dortmund and Wittenberg ($\chi^2 = 3.4141$, $p = 0.33$), Thus, the two bladder cancer groups were combined to one group.

On the basis of the literature, the *GSTM1* negative genotype is a genetically based risk factor for bladder cancer. This was also confirmed in the present study. A remarkable overrepresentation of the *GSTM1* negative phenotype was observed in the bladder cancer patients group compared to the controls who provided a medical history without any malignancy (63 % bladder cancer patients *vs.* 50 % controls). The difference was statistically significant ($\chi^2 = 11.1412$, $p = 0.01$). Furthermore, the *GSTM1*A* allele was more frequently observed in the controls (31 % in controls *vs.* 20 % in bladder cancer patients) leading to a lower risk of bladder cancer ($OR_{total} = 0.5$, $CI_{total} = [0.3 – 0.8]$ for *GSTM1*A/*A* or *GSTM1*A/*0*) whereas the *GSTM1*B* showed no significant difference ($OR_{total} = 0.9$, $CI_{total} = [0.5 – 1.5]$ for *GSTM1*B/*B* or *GSTM1*B/*0*). Due to the low numbers of *GSTM1*A/*B* genotypes a reduced risk could not be confirmed significantly ($OR_{total} = 0.4$, $CI_{total} = [0.1 – 1.0]$) for *GSTM1*A/*B.*

Table 9: *GSTM1* genotype in the investigated groups

***GSTM1* genotype**	***0/*0**	***A/*0 o. *A/*A**	***B/*0 o. *B/*B**	***A/*B**	**Total**
Group					
Controls Surg Do	88 (50%)	54 (31%)	24 (14%)	10 (6%)	176
TCC Ca Witt + TCC Ca Do	184 (63%)	58 (20%)	43 (15%)	8 (3%)	293

An association between *GSTM1* genotype and age or gender was not found. An increased occurrence of the *GSTM1* negative genotype in smokers and ex-smokers could not be found ($\chi^2 = 2.9830$, $p = 0.81$). A total of 67 % in smokers and 71 % in non-smokers presented the *GSTM1* negative genotype. Furthermore, no relevant association between the *GSTM1* genotype and number of pack years, age at the beginning of smoking and duration of having quitted smoking in ex-smokers could be observed.

3.3 Glutathione S-transferase M3 genotype

Distribution in the investigated groups

The portion of the investigated *GSTM3* genotypes in the investigated groups was approximately the same. There was no evidence for the association of a particular *GSTM3* genotype with bladder cancer. In the wide majority of cases in all three investigated groups, the *GSTM3*A/*A* genotype was detected (controls Dortmund: 74 %, bladder cancer patients Wittenberg: 75 %, bladder cancer patients Dortmund: 78 %). Only very few patients presented the homozygous *GSTM3*B/*B* genotype (2 % *vs.* 1 % *vs.* 1 %)

GSTM3 genotype and age

An association between the *GSTM3* genotype and age or gender was not observed.

Table 10: *GSTM3* genotype in the investigated groups

***GSTM3* genotype**	***A/*A**	***A/*B**	***B/*B**	**Total**
Group				
Controls Surg Do	130 (74%)	43 (24%)	3 (2%)	176
TCC Ca Witt	158 (75%)	50 (24%)	2 (1%)	210
TCC Ca Do	65 (78%)	17 (21%)	1 (1%)	83
TCC Ca Witt + TCC Ca Do	223 (76%)	67 (23%)	3 (1%)	293

Table 11: *GSTM3* genotype in dependence of the *GSTM1* genotype in bladder cancer patients from Wittenberg

***GSTM3* genotype**	***A/*A**	***A/*B**	***B/*B**	**Total**
*GSTM*1 genotype				
*0/*0	107 (85%)	18 (14%)	1 (1%)	126
*A/*0 or *A/*A	21 (47%)	23 (51%)	1 (2%)	45
*B/*0 or*B/*B	27 (79%)	7 (21%)	0	34
*A/*B	3 (60%)	2 (40%)	0	5

Table 12: *GSTM3* genotypes in dependence of the *GSTM1* genotypes in controls from Dortmund

***GSTM3* genotype**	***A/*A**	***A/*B**	***B/*B**	**Total**
GSTM1 genotype				
*0/*0	78 (87%)	9 (10%)	1 (1%)	88
*A/*0 or *A/*A	28 (52%)	24 (44%)	2 (4%)	54
*B/*0 or *B/*B	21 (88%)	3 (13%)	0	24
*A/*B	3 (30%)	7 (70%)	0	10

Table 13: *GSTM3* genotype in dependence of the *GSTM1* genotype in bladder cancer patients from Dortmund

***GSTM3* genotype**	***A/*A**	***A/*B**	***B/*B**	**Total**
GSTM1 genotype				
*0/*0	48 (83%)	10 (17%)	0	58
*A/*0 or *A/*A	6 (46%)	6 (46%)	1 (8%)	13
*B/*0 or *B/*B	9 (100%)	0	0	9
*A/*B	2 (67%)	1 (33%)	0	3

GSTM3 genotype and smoking

In bladder cancer patients from Dortmund, 80 % of the smokers as well as 80 % of the ex-smokers presented the *GSTM3*A/*A* genotype. This genotype was presented by 71 % of the non-smokers. Relevant differences were not observed ($\chi^2 = 2.1261$, $p = 0.71$).

Combination of ***GSTM1*A*** und ***GSTM3*B***

A combination of the *GSTM1*A* allele and the *GSTM3*B* allele was observed in all three investigated collectives (bladder cancer patients Dortmund: $\chi^2 = 14.4059$, $p = 0.03$; bladder cancer cases Wittenberg: $\chi^2 = 27.4385$, $p = 0.0001$; controls Dortmund: $\chi^2 = 37.3806$, $p < 0.0001$). An elevated portion of the *GSTM3*A/*B* genotype as well as of the *GSTM3*B/*B* genotype was observed in patients who presented the *GSTM1*A/*A* or the *GSTM1*A/*0* genotype.

4 Discussion

The definite mechanism which influences the development of bladder cancer has not been known so far. To date, polycyclic aromatic hydrocarbons (pah) as well as different aromatic amines which are proven carcinogens in humans like 2-naphthylamine and 4-aminobiphenyl (DOLIN ET AL., 1991; JOHANNSSON ET AL., 1997) are of particular interest.

In this study, the contribution of tobacco smoking to an elevated bladder cancer risk could be confirmed. In bladder cancer patient groups, the portions of smokers and ex-smokers were clearly higher than in the control group (OR = 3.7 smokers and OR = 2.2 ex-smokers from Dortmund; OR = 2.1 smokers and OR = 2.6 ex-smokers from Wittenberg). Regarding the valuation of the high percentages of ex-smokers in the investigated bladder cancer groups (40 % ex-smokers in bladder cancer patients from Dortmund and 45 % ex-smokers in the bladder cancer patients from Wittenberg compared with 35 % ex-smokers in the control group from Dortmund), it must be taken into account that the present study was designed as a retrospective study where the patients were surveyed for their smoking habits after the diagnosis of bladder cancer was disclosed. Thus, it should be taken into account that some patients might have quitted smoking on the occasion of the first symptoms of the disease or at the time the diagnosis was disclosed.

An opposed trend in the number of reported pack years was observed in bladder cancer patients from Dortmund and Wittenberg. Overall 26 % of the bladder cancer patients from Wittenberg reported on 0-10 pack years. With increasing numbers of pack years, the percentage was decreasing almost arithmetically. Only 13 % of the bladder cancer patients reported on more than 40 pack years. In contrast, 2 % of the bladder cancer patients from Dortmund reported on 0-10 pack years and as many as 49 % reported on more than 40 pack years.

It should be clearly kept in mind that in the former German Democratic Republic selling of cigarettes was restricted. Cigarettes and other tobacco products were only available by vouchers.

The observed differences in the smoking habits might be also due to the different cigarette brands available in the former German Democratic Republic and in the Federal Republic of Germany.

It should be noted that the type of tobacco and its processing, particularly the drying, may affect the load of the tobacco smoke with toxicants to a considerable extent. There are some indications which point to a higher bladder cancer risk for smokers having consumed “black”, i.e. air-cured, tobaccos than for those who have consumed “blond”, i.e. flue-cured tobaccos (CLAVEL ET AL., 1989).

Cigarettes in the former German Democratic Republic contained up to 2 mg nicotine and up to 24 mg condensate – almost twice as much as most of the cigarettes in the Federal Republic of Germany (FINSTERBUSCH, 1997). It must be considered that some patients who were prone to suffer a bladder cancer due to tobacco smoking at higher age, might have died earlier due to other tobacco-related diseases. Further studies will be needed to investigate possible differences in bladder cancer risks due to cigarette brands, origin of tobacco, processing of tobacco and so on.

Some studies revealed bladder cancer risks in persons who had early in their life started tobacco smoking (ZEEGERS ET AL., 2002; SADETZKI ET AL., 1999) and put the hypothesis forward that DNA damage early in the life might be compensated worse.

In the present study, such an association could not be observed. The large majority in smokers actually reported on having started tobacco smoking before they turned 20 (Dortmund 76 %, Wittenberg 67 %), but this was in line with the large majority of the smokers in the control group that have also started as teenagers (87 %).

In addition, in the present study the impact of the polymorphic glutathione S-transferases *GSTM1* und *GSTM3* regarding the susceptibility for bladder cancer was investigated.

In the present study, the portion of the *GSTM1* negative bladder cancer patients was
63 %, in contrast to only 50 % in the investigated controls. The difference is statistically significant.

BROCKMÖLLER ET AL. (1994) reported an underrepresentation of *GSTM1*A* carriers in GSTM1 positive bladder cancer patients. Only 23.3 % of the bladder cancer patients but 33.5 % of the controls presented the *GSTM1*A* allele. The authors assumed that the *GSTM1*A* allele might provide a protective effect regarding to the development of bladder cancer.

The results observed in the present study point to the same direction. In 31 % of the controls, the *GSTM1*A* allele was detected, whereas this allele was only observed in 20 % of the bladder cancer patients leading to an odds ratio of 0.5. A sound explanation why the *GSTM1*A* allele, but not the *GSTM1*B* allele, might provide a protective effect regarding to bladder cancer cannot be given at this stage. There is no evidence for differences in enzyme activity and/or substrate specificy between the proteins these two alleles code for (HAYES ET AL., 1989; WIDERSTEN ET AL., 1991). Only differences in the isoelectric point have been observed in the proteins. Possibly, differences in the steric structure of the different GSTM1 isoenzymes or different binding affinities to substrates which have not been characterized to date might be relevant.

ENGEL ET AL. (2002) reported in a meta-analysis of 10 studies, which included 2,149 bladder cancer patients and 1,444 controls, no statistically significant association between smoking state, *GSTM1* genotype and bladder cancer. Nevertheless, it should be noted that the *GSTM1* negative genotype was more often observed in smokers.

In the present bladder cancer group from Dortmund, 67 % of the smokers, 73 % of the ex-smokers and 71 % of the non-smokers revealed the *GSTM1* negative genotype. The high percentage of the *GSTM1* negative genotype in non-smokers with bladder cancer is striking.

In *GSTM1* negative bladder cancer patients from Wittenberg, patients reporting to be a smoker were slightly overrepresented (67 %), compared to ex-smokers (58 %) and non-smokers (55 %) (χ^2 = 8.6052, p = 0.2). Particularly the results from the patients from Dortmund confirm the assumption that the *GSTM1* negative genotype is a risk factor for bladder cancer not primarily related to smoking habits. Occupational and/or environmental exposures to toxicants must be seriously taken into account.

A relevant impact of the *GSTM3* genotype was not observed for any of the investigated parameters which commonly are used to characterize smoking habits like number of pack years, age at beginning of smoking, or duration of having quitted smoking in ex-smokers. Thus it can be concluded that the *GSTM3* state did not modulate the bladder cancer risk in the two investigated bladder cancer groups.

References

Arbeitsgemeinschaft bevölkerungsbezogener Krebsregister in Deutschland. 3. erweiterte, aktualisierte Ausgabe, Saarbrücken, 2002

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

Black RJ**,** F. Bray F, Ferlay J, Parkin DM (1997) Cancer incidence and mortality in the European Union. Cancer Registry data and estimates of national incidence for 1990. Eur J Cancer **33**,1075-1107

Brockmöller J, Kerb R, Drakoulis N, Staffeldt B, Roots I (1994) Glutathione S-transferase M1 and its variants A and B as host factors of bladder cancer susceptibility: a case-control study. Cancer Res **54**, 4103-4111

Cantlay AM, Smith CA, Wallace WA, Yap PL, Lamb D, Harrison DJ (1994) Heterogeneous expression and polymorphic genotype of glutathione S-transferases in human lung. Thorax **49**, 1010-1014

Chasseaud (1979) The role of glutathione and glutathione S-transferases in the metabolism of chemical carcinogens and other electrophilic agents. Adv Cancer Res **29,** 175-184

Clavel J, Cordier S, Boccon-Gibbod L, Hemon D (1989) Tobacco and bladder cancer in males: increased risk for inhalers and smokers of black tobacco. Int J Cancer **44**, 605-610

Dolin PJ (1991) An epidemiological review of tobacco use and bladder cancer. J Smoking Related Dis **2,** 129-143

Engel LS, Taioli E, Pfeiffer R, Garcia-Closas M, Marcus PM, Lan Q, Boffetta P, Vineis P, Autrup H, Bell DA, Branch RA, Brockmöller J, Daly AK, Heckbert SR, Kalina I, Kang D, Katoh T, Lafuente A, Lin HJ, Romkes M, Taylor JA, Rothman N (2002) Pooled analysis and meta-analysis of glutathione S-transferase M1 and bladder cancer: a HuGE review. Am J Epidemiol **156**, 95-109

Finsterbusch S (1997) Für westdeutsche Lungen einfach zu kräftig. FAZ **32**, 17

Fjellstedt TA, Allen RH, Duncan BK, Jacoby WB (1973) Enzymatic conjugation of epoxides with glutathione. J Biol Chem **248,** 3702-3707

Golka K, Kopps S, Myslak ZW (2004) Carcinogenicity of azo colorants: influence of solubility and bioavailability. Toxicol Lett **151**, 203-210

Habig WH, Pabst MJ, Jacoby WB (1974) Glutathione S-transferases – The first enzymatic step in mercapturic acid transformation. J Biol Chem **249**, 7130-7139

Hayes JD, Kerr LA, Cronshaw AD (1989) Evidence that glutathione S-transferases B1B1 and B2B2 are the products of separate genes and that their expression in human liver is subject to inter-individual variation. Molecular relationships between the B1 and B2 subunits and other Alpha class glutathione S-transferases. Biochem J **264**, 437-445

Inskip A, Elexperu-Camiruaga J, Buxton N, Dias PS, MacIntosh J, Campbell D, Jones PW, Yengi L, Talbot JA, Strange RC, Fryer AA (1995) Identification of polymorphism at the glutathione S-transferase, GSTM3 locus: evidence for linkage with GSTM1*A. Biochem J **312**, 713-716

International Agency for Research on Cancer (IARC) (Ed): Tobacco smoke and involuntary smoking. World Health Organisation, Lyon, 2004 (IARC Monographs on the Evaluation of the Carcinogenetic Risk of Chemicals to Humans, Vol 83)

Ishigaki S, Abromowitz M, Listowsky I (1989) Glutathione S-transferases are major cytosolic thyroid hormone-binding proteins. Arch Biochem Biophysic **273**, 265-272

Johannsson SL, Cohen SM (1997) Epidemiology and etiology of bladder cancer. Semin Surg Oncol **13**, 291-298

Kempkes M, Golka K, Reich S, Reckwitz T, Bolt HM (1996) Glutathione S-transferase GSTM1 and GSTT1 null genotypes as potential risk factors for urothelial cancer of the bladder. Arch Toxicol **71**, 123-126

Loktionov A, Watson MA, Gunter M, Stebbings WSL, Speakman CTM, Bingham SA (2001) Glutathione-S-transferase gene polymorphisms in colorectal cancer patients: interaction berween GSTM1 and GSTM3 allele variants as a risk-modulating factor. Carcinogenesis **22**, 1053-1060

Mannervik B, Danielson UH (1988) Glutathione transferases – structure and catalytic activity. CRC Critical Reviews in Biochemistry **23**, 283-337

Myslak, ZW, Bolt HM (1988) Berufliche Exposition gegenüber Azofarbstoffen und Harnblasenkarzinom-Risiko. Zbl. Arbeitsmed. **38**, 310-321

Negri E 1, La Vecchia C (2001) Epidemiology and prevention of bladder cancer. Eur J Cancer Prev **10**, 7-14

Pacifici GM, Warholm M, Guthenberg C, Mannervik B, Rane A (1987) Detoxification of styrene oxide by human liver glutathione transferase. Hum Toxicol **6**, 483-489

Raney KD, Meyer DJ, Ketterer B, Harris TM, Guengerich FP (1992) Glutathione conjugation of aflatoxin B1 exo- and endo-epoxides by rat and human glutathione S-transferases. Chem Res Toxicol **5**, 470-478

Robertson IG, Guthenberg C, Mannervik B, Jernström B (1986) Differences in stereoselectivity and catalytic efficiency of three human glutathione transferases in the conjugation of glutathione with 7 beta,8 alpha-dihydroxy,9 alpha,10 alpha-oxy-7,8,9,10-tetrahydrobenzo(a)pyrene. Cancer Res **46**, 2220-2224

Schmidt T (2005) Die polymorphen Glutathion-S-Transferasen M1 und M3 als Suszeptibilitätsfaktoren beim Harnblasenkarzinom. Dissertation, Medizinische Fakultät der Ruhr-Universität Bochum

Schnakenberg E, Breuer R, Werdin R, Dreikorn K, Schloot W (2000) Susceptibility genes: GSTM1 and GSTM3 as genetic risk factors in bladder cancer. Cytogenet Cell Genet **91**, 234-238

Widersten M, Pearson WR, Engstrom A, Mannervik B (1991) Heterologous expression of the allelic variant mu-class glutathione transferases mu and psi. Biochem J **276**, 519-524

Yengi L, Inskip A, Gilford J, Alldersea J, Bailey L, Smith A, Lear JT, Heagerty AH, Bowers B, Hand P, Hayes JD, Jones PW, Strange RC, Fryer AA (1996) Polymorphism at the glutathione S-transferase locus GSTM3: interactions with cytochrome P450 and glutathione S-transferase genotypes as risk factors for multiple cutaneous basal cell carcinoma. Cancer Res **56**, 1974-1977

Zeegers MPA, Goldbohm RA, van den Brandt PA (2002) A prospective study on active and environmental tobacco smoking and bladder cancer risk (the Netherlands). Cancer Causes Control **13**, 83-90

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