Urinary bladder cancer is a socially significant health care problem. A diverse array of aromatic and heterocyclic amines, derived from the chemical and transport industry, diet, and cigarette smoke are considered carcinogens for the bladder. To exert their carcinogenic effect and to initiate the carcinogenic response, the arylamines require a metabolic activation by the host enzymes to chemically reactive compounds. The aim of this article was to review the latest and basic research developments on the role of the polymorphisms in the carcinogen metabolizing enzymes N-acetyltransferase (NAT), Glutathione S-transferases (GST), and Soluble sulfotransferases (SULT), with emphasis on the susceptibility to urinary bladder cancer. A PubMed search was conducted to identify original and review articles containing information about these polymorphic variants in different populations and according to their prevalence in bladder cancer patients.

We noticed that some genotypes were found to be predisposing and some protective for bladder cancer development. The NAT2 slow genotype, together with GSTM1 null genotype facilitated the development of bladder cancer in almost all ethnic groups. The 213His allele of the SULT1A1 gene which is associated with lower enzyme activity and decreased mutagen activation was reported to protect from bladder cancer in almost all studies.

Key words: bladder, cancer, pharmacogenetic, population, risk
1976 and 2014. Figure 1 summarizes the number of studies identified, reasons for exclusion and the profile of the full-length articles included in this review.

**Carcinogen metabolizing enzymes**

NAT is an enzyme the activity of which leads to detoxification of aromatic amines. It is coded by two genes: NAT1 and NAT2 [7]. Each of them has genetic variants which encode for either rapid or slow acetylation. The NAT 2 enzyme activity is reduced in individuals with two slow-acetylator alleles, which genotype predominates in white population [8,9]. The risk of bladder cancer morbidity in such individuals is higher than in individuals with rapid acetylation [10]. Enzymological and genetic investigations of the acetyltransferase gene show that it plays a role in both activation and detoxification of arylamines and their congenials. In the liver, arylamine metabolism includes two alternative pathways: N-acetylation by NAT2 or N-hydroxylation by CYPIA2. N-acetylation by NAT2 leads to arylamine deactivation, and formation of non-reactive compounds (Figure 2). N-hydroxylation by CYPIA2 leads to formation of the hydroxylamine. This hydroxylated form is transported to the bladder and metabolized by NAT1 (highly expressed in bladder epithelium) to a highly reactive species that can form DNA adducts [11]. Alleles with decreased activity in the liver (NAT slow acetylators) as well as alleles with increased activity in the bladder (NAT1 rapid acetylators) are considered to increase the carcinogenic potential of arylamines [12,13].

GSTs are metabolizing enzymes of phase II that protect normal cells by the detoxification of carcinogens, toxins, drugs, and products of the oxidative stress by glutathione conjugation [14] (Figure 3). The GST enzyme family includes 5 cytosolic isoforms GST-α (GSTA), μ(GSTM), π(GSTP), θ(GSTT), and σ(GSTS). The GSTT1, P1, and M1 isoforms are polymorphic, and particular allelic variants have been suggested to increase or decrease the risk in the development of uroepithelial malignancies [15]. It is thought that upregulation of different classes of various GST in the bladder transitional cell carcinoma (TCC) influences the TCC growth by inhibition of apoptosis and by providing a reduced cellular environment [16].

SULTs are important enzymes in the elimination of various xenobiotics, and the bioactivation of dietary and other bladder carcinogens, such as heterocyclic amines (Figure 2). A functional polymorphism in the SULT1A1 gene (SULT1A1* - Arg215His substitution) is deemed related with the susceptibility to a variety of cancers as well as mutagenicity following exposure to arylamines from cigarette smoke and other environmental toxins [17]. Although statistically significant associations were observed between the SULT1A1* genotype and mammary, pulmonary, esophageal,
Table 1. Investigated odds ratio of cancer risk among people from different ethnicities, according to the NAT, GST and SULTs genotype/phenotype

<table>
<thead>
<tr>
<th>First author [Ref]</th>
<th>Population</th>
<th>Cases</th>
<th>Controls</th>
<th>Cancer risk - OR (95%CI)</th>
<th>Enzyme genotype/phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hayes [24]</td>
<td>Chinese</td>
<td>38</td>
<td>43</td>
<td>0,7 (0,1-4,5)</td>
<td>NAT2 slow acetylator - low benzidine exposure</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0,6 (0,1-3,5)</td>
<td>NAT2 slow acetylator - medium benzidine exposure</td>
</tr>
<tr>
<td>Song [25]</td>
<td>Chinese</td>
<td>208</td>
<td>212</td>
<td>1,64 (1,11–2,42)</td>
<td>GSTM1 null</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1,72 (1,00–2,95)</td>
<td>GSTM1/GSTT1 - double null</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Bladder cancer 1,81 (1,12–2,93)</td>
<td>GSTM1 null</td>
</tr>
<tr>
<td>Kim [26]</td>
<td>Korean</td>
<td>113</td>
<td>221</td>
<td>0,85 (0,54-1,55)</td>
<td>GSTT1 null</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6,79 (0,67- 68.82)</td>
<td>NAT2 slow acetylator among smokers</td>
</tr>
<tr>
<td>Lee [27]</td>
<td>Korean</td>
<td>232</td>
<td>165</td>
<td>1,3 (0,9–2,0)</td>
<td>GSTM1 null</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2,2 (1,2–4,3)</td>
<td>GSTM1 null/GSTT1 null</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Bladder cancer 1,00 (0,46–2,16)</td>
<td>CYP2D6 heterozygous</td>
</tr>
<tr>
<td>Sobti [28]</td>
<td>Indian</td>
<td>100</td>
<td>76</td>
<td>2,90 (0,76–11,10)</td>
<td>CYP2D6 heterozygous and GSTT1 null</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2,53 (1,17–5,46)</td>
<td>GSTT1 null</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Bladder cancer 1,71 (1,05–2,79)</td>
<td>GSTM1 null</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2,62 (1,56–5,05)</td>
<td>GSTM1 null and GSTT1-positive</td>
</tr>
<tr>
<td>Katoh [29]</td>
<td>Japanese</td>
<td>145</td>
<td>145</td>
<td>1,25 (0,62–2,51)</td>
<td>GSTP1G genotype (GSTP A/G or GSTP G/G)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Oral cancer 1,95 (1,05-3,58)</td>
<td>GSTP1G</td>
</tr>
<tr>
<td>Katoh [30]</td>
<td>Japanese</td>
<td>47</td>
<td>122</td>
<td>Lung cancer 1,18 (0,52-2,58)</td>
<td>GSTP1G</td>
</tr>
<tr>
<td></td>
<td></td>
<td>140</td>
<td>122</td>
<td>Gastric 1,56 (0,9-2,73)</td>
<td>GSTP1G</td>
</tr>
<tr>
<td></td>
<td></td>
<td>103</td>
<td>122</td>
<td>Colon 1,58 (0,88-2,87)</td>
<td>GSTP1G</td>
</tr>
<tr>
<td></td>
<td></td>
<td>106</td>
<td>122</td>
<td>Urothelial 1,47 (0,78-2,80)</td>
<td>GSTP1G</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Bladder cancer 1,37 (1,01-1,87)</td>
<td>GSTM1 null</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3,09 (1,69-5,63)</td>
<td>NAT2 slow</td>
</tr>
<tr>
<td>Tsukino [32]</td>
<td>Japanese</td>
<td>325</td>
<td>325</td>
<td>1,48 (1,01-2,15), in relation of smoking</td>
<td>GSTM1 null</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4,28 (1,96-9,36), in relation of smoking</td>
<td>NAT2 slow acetylator</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Bladder cancer 2,45 (1,04-5,98)</td>
<td>SULT1A1*1 ((213)Arg) and NAT2 slow acetylator</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2,07 (0,48–9,84)</td>
<td>SULT1A1*2 ((215)His) and NAT2 slow acetylator</td>
</tr>
<tr>
<td>Ozava [18]</td>
<td>Japanese</td>
<td>166</td>
<td>214</td>
<td>Bladder cancer 0,67 (0,45–1,05)</td>
<td>SULT1A1 ((213)His)</td>
</tr>
<tr>
<td>Hung [33]</td>
<td>Caucasian - Italian</td>
<td>201</td>
<td>214</td>
<td>Bladder cancer 0,67 (0,45–1,05)</td>
<td>SULT1A1 ((213)His)</td>
</tr>
<tr>
<td>Wu [22]</td>
<td>Taiwanese</td>
<td>187</td>
<td>308</td>
<td>Esophageal cancer 3,53 (2,12-5,87)</td>
<td>SULT1A1 ((213)His)</td>
</tr>
<tr>
<td>Roupret [34]</td>
<td>Caucasian - French</td>
<td>268</td>
<td>268</td>
<td>Urothelial cell carcinomas 2.18 (1,28-3,69)</td>
<td>SULT1A1*2</td>
</tr>
</tbody>
</table>

Continued on next page
and urothelial cancer [18,19], there are altogether few studies on the SULT1A1 and the results are inconsistent [18,20-22].

Polymorphic enzyme variants related to the bladder cancer susceptibility in different ethnic groups (Table 1)

The incidence of bladder cancer varies in different ethnic groups. It is highest in Southern Europe and lowest in Western Africa [23]. This could be due to the different environmental factors, as well as to different genetic predisposition in these population groups.

### Asia

Many studies on the Chinese, Japanese, Korean, and Indian populations have been published. In 1993, Hayes et al. investigated the Chinese
Bladder cancer and carcinogen metabolizing enzymes

populations occupationally exposed to benzidine and found no increase in the bladder cancer risk for the slow N-acetylation phenotype or for slow N-acetylation-associated double mutations in NAT2 [24]. No interaction between this genotype and benzidine exposure was observed. This can be explained with the fact that benzidine is a much better substrate for NAT1 than NAT2 [24]. However, increased risk and a higher grade of differentiation of bladder cancer were associated with the NAT2 slow-acetylator genotype, GSTM1 null and GSTM1/GSTT1 - double null alleles [25].

Figure 2. Simplified scheme of arylamine metabolism pathway. Arylamines are N-acetylated by NAT2 in the liver, transforming them to relatively nonreactive. Alternatively, they may be N-hydroxylated by CYP1A2, transported to the bladder, and undergo O-acetylation by NAT1, to form a highly reactive species.

Figure 3. Simplified scheme of action of the enzymes glutathione S-transferases (GSTs) and soluble sulfotransferases (SULTs) in the detoxification of polycyclic aromatic hydrocarbons (PAH).
Moreover, among the studied P450 variants, the carriers of at least one CYP2A6*4 allele showed a lower risk of bladder cancer development than the non-carriers. CYP2A13 was not associated with an increased risk or unfavorable tumor characteristics [25]. In a similar study in Korean population, an increased risk for bladder cancer was found among the patients with a history of tuberculosis and bronchial asthma, carriers of a combination of rapid acetylator genotypes and either GSTM1 null or GSTT1 null genotypes [26] (the smoking history turned out to be insignificant). Similarly to the Chinese population, GSTM1 null genotype was found to be a significant risk factor for bladder cancer, whereas GSTT1 and slow acetylator genotypes were insignificant. In 2000, Kim et al. could not find any association between GSTM1, GSTT1, slow acetylator genotypes, and bladder cancer risk among smokers [26]. However in the following study performed in South Korea, a strong association was found between GSTM1 null genotype and bladder cancer risk [27]. Another study from India indicated a 3-fold increase in the risk of developing bladder cancer in the presence of GSTT1 null and one copy of the variant CYP2D6 allele, while there was no association between the heterozygous genotype of the CYP2D6 gene with risk of bladder cancer. Subjects with the GSTM1 null genotype had a slightly significant association with the bladder cancer risk which increased to 2.5-fold in the presence of the GSTT1 null genotype [28]

The opposite was reported in Japanese patients, where urothelial cancer risk was increased due to the GSTM1 null genotype, especially among smokers. The individuals with a combination of GSTM1 null genotype and GSTT1 positive genotype had a 2-fold risk compared with the GSTT1 null genotype [29].

Another GST variant - GSTP1 - is involved in the inactivation of cigarette smoke carcinogens. Sequence variation in the gene may alter the bladder cancer susceptibility. The study of the GSTP1 AG polymorphism (which reduces the catalytic activity of the GSTP1 enzyme) in Japanese patients with different types of smoking-related cancers, showed no difference between smoking patients and controled individuals for the frequency of the GSTP1 AG polymorphism for any cancer [30]. In 2007, Kellen et al. investigated the association between GSTP1 Ile105Val and bladder cancer risk. GSTP1 Ile105Val appeared to be associated with a modest increase in the risk of bladder cancer and the association turned to be the strongest in Asian countries [31].

Unlike the previous described studies, Tsukino et al. (2004) showed that the GSTM1 null and NAT2 intermediate or slow genotype are associated with increased risk of urothelial cancer in relation to smoking amount [32]. In this case – control study in Japanese population, the frequencies of GSTM1 null and NAT2 slow genotypes were found to be significantly higher in the cases compared to controls [32].

In 2002, Ozawa et al. performed combined analyses of different alleles of carcinogenic aromatic amine-activating phase II enzymes [18]. The highest risk for urothelial cancer was shown for the combination of SULT1A1 and NAT2 slow genotypes. Additionally, the wild-type SULT1A1 ((213) Arg) alleles were slightly overrepresented in both smoking and nonsmoking urothelial cancer patients compared to SULT1A1*2 ((213) His) allele, which is in agreement with the study of Hung et al. [33]. However SULT1A1 ((213) His) allele was associated with statistically significantly increased risks of esophageal cancer in Taiwan, lung cancer in USA, and upper urinary tract urothelial cell carcinomas in French patients [20,22,34].

Africa

In the Tunisian population it was found that NAT2 slow acetylator individuals carrying wild-type GSTT1 or GSTM1 null genotypes had a higher risk for bladder cancer. This effect increased for smokers, harboring slow or an intermediate NAT2, wild-type GSTT1, and GSTM1 null genotypes, compared to non-smokers carrying rapid NAT2, wild-type GSTM1 and GSTT1 null genotypes. Among the NAT2 slow acetylator genotype, the NAT2*5/*7 diplotype was reported to have a highest risk for bladder cancer development [35].

Europe

NAT2 enzyme activity is reduced in about 50% of Europeans, and in 1982 Cartwright et al. suggested that acetylator status could be used to identify susceptible individuals in potentially hazardous occupations [36].

This finding was confirmed by Risch et al. in 1995 who reported an excess of slow acetylators in bladder cancer patients with a history of smoking or occupational exposure to aromatic amines [37].

Lower et al. in 1979 examined the possible correlations between N-acetyltransferase pheno-
type and urinary bladder cancer risk in rural and urban populations. Urban urinary bladder cancer patients from Denmark displayed a 13% excess of individuals with the slow acetylator phenotype when compared to a control group [10]. In rural population from Sweden, where bladder cancer incidence is lower than in urban, no difference in slow acetylator distribution was observed between bladder cancer and control populations. The latter was thought to be due to relative lack of involvement of arylamines in the etiology of rural bladder cancer. In 1997, Okkles et al. reported that NAT1 and NAT2 allele frequencies were not significantly different between the cases and controls in a Danish population [38]. An association between the NAT2 slow genotype and bladder cancer risk existed only among smokers. In this group a higher frequency of the mutant NAT2 allele and a corresponding lower frequency of the wild-type NAT2 allele was shown among the cases compared with the controls [38]. Although about 50% of Caucasians have deletion of the two copies of the gene coding for GSTM1 (GSTM1 0/0 genotype) and have been shown to be at higher risk of bladder cancer, Okkles et al. (1997) found no association of the NAT1 and GSTM1 genotypes with bladder cancer risk among smokers [38-40]. Furthermore, they thought that combinations of the NAT2 and GSTM1 genotypes were not risk factors of bladder cancer, and normal NAT1/fast NAT2 seems to be a protective genotype combination compared with all other NAT1/NAT2 genotype combinations.

One of the NAT1 allele variants - NAT1*10 - is thought to be a rapid acetylator allele associated with an increase in the N-acetyltransferase activity in bladder, colon, liver, and erythrocytes, and an increase of carcinogen-DNA binding adduct in the urinary bladder. NAT1*10 allele is responsible for the higher levels of metabolic activation of N-hydroxy-aromatic amines in human urinary bladder cytosol and human uroepithelial cells [41]. However, in the study of Gu et al. (2005) with 507 Caucasian bladder cancer patients and 513 age-, gender-, and ethnicity-matched healthy controls, no significant association between NAT1*10 allele and bladder cancer risk was found [42]. According to the NAT2 slow acetylator genotypes their results confirmed the studies of Cartwright et al. (1982), Risch et al. (1995) and Okkles et al. (1997) that these genotypes are risk factors for bladder cancer, particularly in smokers and older individuals [36-38]. Heavy smokers with NAT2 slow acetylator genotypes showed an over 6-fold increase in bladder cancer risk compared to smokers with NAT2 rapid acetylator genotypes [42].

In a case-control study that included 89 TCC Greek patients and 147 controls, a higher frequency of slow acetylator genotypes was found in the patient group. Among them, 341C/341C homozygotes and 341C/857A compound heterozygotes had the most excessive risk for bladder cancer. The 341C/341C homozygotes were reported to have a higher risk for more aggressive disease [43]. In another Mediterranean region (Spain) Garsia-Closas et al. (2005) showed that the GSTM1 null genotype increased the overall risk of bladder cancer, and the NAT2 slow-acetylator genotype increased the risk, particularly among cigarette smokers [8]. These polymorphisms could account for up to 31% of bladder cancers because of their high prevalence, although the relative risks were modest.

In 2013, Matic et al. found no significant difference in the distributions of GSTM1, GSTT1, GSTA1, and GSTP1 gene variants between patients and controls in their hospital-based case-control study [44]. Significant association with bladder cancer risk was found for lower activity GSTA1 AB/BB and GSTM null genotype in smokers compared to GSTA1 AA and GSTM1 active non-smokers.

Hung et al. in their study showed that 213His allele of the SULT1A1 gene was associated with lower enzyme activity and decreased mutagen activation, that might result in a protective effect on bladder carcinogenesis [33]. However, the results based on a male population of Northern Italy, showed that the 213His allele of the SULT1A1 gene was associated with a moderately reduced risk of bladder cancer [33]. Opposite results were reported for the SULT1A1 (213His) allele in a population of French Caucasian patients, where it was associated with statistically significantly increased risks of upper urinary tract urothelial cell carcinomas [34].

North America

The role of N-acetylation polymorphisms in smoking-associated bladder cancer was evaluated by Taylor et al. in 1998 [45]. They found no association between the studied NAT2 genotypes (NAT2*4, NAT2*5, NAT2*6, NAT2*7, NAT2*14) and bladder cancer risk whether the genotypes were considered alone or in combination with smoking. They demonstrated increased bladder cancer risk for individuals carrying the NAT1*10 allele among smokers. The highest risk was observed...
for patients homozygous for the NAT1*10 allele. The authors also showed that bladder cancer risk from smoking exposure is particularly high in those who inherit NAT2 slow alleles in combination with one or two copies of the NAT1*10 allele. In 2004, Castelao et al. reported that NAT1-rapid, NAT2-rapid, and CYP1A2-rapid genotype/phenotype influence the protective effect of carotenoids on bladder cancer in non-Asians of Los Angeles, California [46]. Later, Yuan et al. (2008) found no associations between bladder cancer risk and NAT1 genotype or CYP1A2 phenotype, but reported a strong association for GSTT1 and NAT2 slow acetylation among individuals with known high exposures to carcinogenic arylamines [47].

Bell et al. (1993) and Muscat et al. (2008) investigated the racial differences in GSTM1, P4501A2 (CYP1A2) and NAT2 genotype frequency in black and whites [39,48]. Bell et al. [39] found that the GSTM1 0/0 genotype occurred less frequently among blacks (35%) than among whites (49%). Muscat et al. calculated that the putative combined low risk phenotype (slow CYP1A2/rapid NAT2) was more common in blacks than in whites (25 vs 15%) [48]. No significant racial differences were observed in slow and rapid CYP1A2 phenotypes, and in the combined slow NAT2/rapid CYP1A2 phenotype.

In the Zheng et al. study (2003) of the Soluble sulfotransferase SULT1A1 gene (213His allele) a statistically significant reduced risk of bladder cancer was observed only in women but not in men with the mutant allele [49]. There was also a reduced bladder cancer risk in never smokers with the mutant allele, but not in former or current smokers.

Conclusion

There is a large amount of data generated from numerous studies with various designs. In each study an assorted enzyme set, isoenzymes or allelic variants were used. The most prevalent genetic variant for Asians which increases the bladder cancer risk is the GSTM1 null genotype. There is no consensus on the effect of the NAT2 slow acetylator genotype. The protective polymorphic variant for bladder cancer development was found to be CYP2A6*4 allele.

In the Tunisian population, genetic variants facilitating the development of bladder cancer are slow NAT2, wild-type GSTT1 and GSTM1 null genotypes. The protective genotypes are rapid NAT2, wild-type GSTM1 and GSTT1 null genotypes.

For for North Americans and Europeans NAT2 slow acetylator and GSTM1 0/0 genotypes are risk factors for bladder cancer and normal NAT1 / fast NAT2 seems to be a protective genotype combination. The lower frequencies of the GSTM1 0/0 and slow NAT2 genotypes in blacks than in whites, and a higher frequencies of low risk phenotype (slow CYP1A2 / rapid NAT2) in blacks, may be offered as an explanation for the observed lower incidence of bladder cancer in Afroamericans.

The 213His allele of the SULT1A1 gene - associated with lower enzyme activity and decreased mutagen activation - is reported to protect from bladder cancer in almost all studies.

All the described genetic variants are only predisposing factors for bladder cancer development. A combination with a high exposure to carcinogenic, such as arylamines, smoking and hazardous occupational exposures is needed to trigger the malignant neoplastic process. That is why we suggest that genotyping for relevant risk polymorphic variants, and regular screening of susceptible individuals working in conditions of well defined carcinogenic exposures might help reduce the incidence, severity and mortality of bladder cancer.

Acknowledgements

This work was supported by the Bulgarian Ministry of Education: DMY 03/48, 12.12.2011 and collaboration project between MANU and BAS.
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