Genetic polymorphism of N-acetyltransferase and glutathione S-transferase related to neoplasm of genitourinary system

Minireview

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Genetically determined risk factors may considerably contribute to the development of neoplastic diseases, including neoplasm of urinary organs, e.g. bladder and prostate cancers. It is believed that they may result, among others, from the differences in the metabolism of environmental carcinogens and mechanisms of DNA repair. There is a clear evidence that the kind and rate of metabolism is genetically determined by polymorphic enzyme coding genes participating in the process of xenobiotic transformation. Genetic polymorphism has been confirmed for a number of enzymes involved in the reaction of oxidation or conjugation of exo- and endogenous xenobiotics. Gene variability may alter the expression or enzymatic activity of coded enzymes. Therefore, the cancer risk assessment should also be based on individual differences in the ability to activate (phase I) or to detoxify (phase II) possible carcinogens.

In the present study, the information on the significance of glutathione S-transferase (GST) and N-acetyltransferase (NAT) gene families in protection of human health and incidence of various diseases is summarized. The role of hereditary polymorphisms of GST and NAT genes involved in the etiology of neoplasm of urinary organs is controversial. That is why, special attention has to be focused on the recent information on a possible role of GST and NAT polymorphisms in the predisposition to urinary bladder, prostate and urothelial transitional cell carcinoma.

Key words: N-acetyltransferase, glutathione S-transferase, cancer of the urinary organs, susceptibility, polymorphism, molecular epidemiology.

Over many years possible relationship between individual hereditary susceptibility, manifested by inherited set of genes coding enzymes, involved in xenobiotic metabolism and the risk of cancer has been the subject of extensive scientific research. Genetic differences observed in the expression and activity of xenobiotic metabolising enzymes and substrate specificity result exactly from the presence of polymorphic alleles coding these enzymes. The differences in the distribution of low-penetrance genes are mainly due to their genetic polymorphism related to the changes in nucleotide sequence, which occurs in at least one percent of the general population.

Most of carcinogenic chemicals are not reactive per se, and require metabolic activation before reacting with the genetic material, which may lead to mutations in the further development of carcinoma. Metabolic activation of chemical compounds proceeds by their oxidation, hydrolysis or reduction, involving phase I enzymes of biotransformation. Phase II enzymes catalyse detoxification of the activated compounds in reactions to conjugation. As a result, very reactive xenobiotics are transformed into compounds, which are easily removed from the organism. Due to the existence of certain gene families, biotransformation enzymes have a number of isoforms. They include cytochrome P450 dependent monooxygenases (CYP), N-
acetyltransferases (NAT) and glutathione S-transferases (GST). Within some of these families, there are several genetic variations, usually caused by deletion or single mutations. Some of genetic polymorphisms have no effect on the enzyme, whilst others have been reflected in the changed catalytic activity of the enzyme, which leads to its increase, loss, or change of substrate specificity. The balance between phase I and II enzymes of xenobiotic biotransformation seems to play a significant role in the potential defence against possible genotoxic effect of certain chemicals and thus against the initiation of carcinogenic processes. As hereditary genetic agents, polymorphic low-penetrance genes coding these enzymes determine individual susceptibility to the effects of xenobiotics and indicate high risk groups [1, 23].

It is believed that individuals with defective NAT and GST genotypes, with GSTMI*0 and GSTT1*0 alleles which have been completely or partly deleted or mutated NAT2*5B or NATI*10 alleles are more susceptible to potentially carcinogenic compounds. This results from the fact that the existence of genetic variations is associated with the changed catalytic activity or substrate specificity of the enzyme. Therefore, the researchers try to find out whether there is a relationship between genetic polymorphisms of xenobiotic metabolising enzymes and neoplasm of genitourinary organs in the following order: prostate, kidneys and urinary bladder [19, 23, 47]. Although the literature data do not confirm this relationship explicitly, primarily due to insufficient size of study and reference groups and/or inappropriate method of their selection, most reports demonstrate a link between NAT and GST genetic polymorphism and cancer of the urinary system.

Molecular genetics of N-acetyltransferases and glutathione S-transferases

N-acetyltransferases, which belong to phase II of xenobiotic transformation are involved in reactions of acetylation of arylamines, components of both carcinogenic compounds and various drugs. In humans two isoforms, NAT1 and NAT2 with different acetylation phenotypes (rapid or slow), characterised by different response to certain drugs are observed. Thus N-acetylation polymorphism determines individual variability in biotransformation of aromatic amines (4-aminobiphenyl), hydrazines, including acetoxy esters, arylamines, and heterocyclic amines (carcinogen responsible for the urinary bladder cancer) present in tobacco smoke and supplied with the diet [15, 22]. In the human genome, three genetic loci have been found, strictly connected with NAT expression. One of them, NATP, seems to be a pseudogene coding a non-functional protein. The nucleotide sequence of the other two expressed genes NAT1 and NAT2 is identical in 87%. Different nucleotide changes occur in NAT sequences: nucleotide substitutions (all NAT2, some NAT1 alleles), insertions or deletions (NATI). So far, 24 different NAT1 alleles and 26 different NAT2 alleles have been found. NAT2*4 and NATI*4 are considered to be wild-type genes coding individual N-acetyltransferases [2, 3, 15, 19, 20, 22]. It is believed that these mutated NAT2 gene variations usually lead to decreased ability to catalyse acetylation (NAT2*5B, NAT2*5A, NAT2*5C, NAT2*6A, NAT2*7B), already confirmed by in vivo studies on acetylation phenotype. In individuals with NATI*10 gene, the efficiency of the N-acetylation reaction is also higher, and consequently they exhibit rapid acetylation phenotype [15, 18, 19, 31] (Tab. 1).

Many ethnic variability in the incidence and mortality from cancer may be due to differences in metabolism and exposure to environmental or dietary carcinogens. It is well known that there are differences in the activity or rate of metabolising chemical compounds in different ethnic groups. Wild-type alleles NAT*4, determining slow acetylation, occur in 75% of the Caucasian populations and there are most common than NATI*10 alleles, responsible for rapid acetylation [37]. Wild-type alleles NAT2*4 are found in 20–25% of Europeans and US Caucasians, while mutated NAT2*5B and NAT2*6A are far more frequently observed [15, 57]. Ethnic frequencies of NAT variant alleles are shown in Table 2.

An important enzyme of phase II of detoxification, GST, plays a considerable role in the defence against the products of oxidative stress and carcinogenic electrophiles. Many studies demonstrate that enzymatic activity of different GST forms exhibits substantial individual differences, which may indicate the presence of genetic polymorphism. Taking into account the biochemical, and structural properties, homologies in the nucleotide sequence and localisation on the human chromosome, the enzymes have been classified as follows: alpha (GSTA), mu (GSTM1, pi (GSTP1), theta (GSTT1), zeta (GSTM1), sigma (GSTS) and kappa (GSTK) [6, 25]. The existence of five genetic GST polymorphisms: GSTMI, GSTM3, GSTP1, GSTT1, GSTZ1 within this multigene family have been confirmed [12]. GSTM1 polymorphism is associated with the existence of 3 alleles, which affect the expression of the enzyme: GSTMI*0, completely or partly deleted, GSTMI*A and GSTMI*B, different only from a single base in the nucleotide sequence. The catalytically active enzyme is coded by GSTMI*A and GSTMI*B genes, and the lack of GSTM1 expression is caused by the deletion of both GSTMI gene copies [34, 36, 49]. GSTT1 genetic polymorphism is related to the deletion of the gene, resulting in the normal GSTT1 (+) and GSTT1 null genotype with the lack of expression of this form of the enzyme and increased risk of cancer in the population [23, 29] (Tab. 1). Due to ethnic variability in the distribution of polymorphic GST isoforms, about a half of the Caucasian population in Western Europe and the USA
Table 1. Genetic polymorphism of GST and NAT enzymes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Nucleotide change</th>
<th>Amino acid change</th>
<th>Consequence of allelic variants</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAT1'4</td>
<td>None</td>
<td>None</td>
<td>Wild-type</td>
</tr>
<tr>
<td>NAT1'10</td>
<td>T^{1088}A, C^{1095}A</td>
<td>None</td>
<td>Slow-acetylation</td>
</tr>
<tr>
<td>NAT1'14</td>
<td>G^{561}A, T^{1088}A, C^{1095}A</td>
<td>Arg^{187}Gln</td>
<td>Elevated NAT1 activity</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Rapid-acetylation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Decrease NAT1 affinity</td>
</tr>
<tr>
<td>NAT2'4</td>
<td>None</td>
<td>None</td>
<td>Wild-type</td>
</tr>
<tr>
<td>NAT2'5B</td>
<td>T^{341}C, C^{383}T, A^{803}G</td>
<td>Ile^{114}Thr, Lys^{268}Arg</td>
<td>Reduction of NAT2 activity</td>
</tr>
<tr>
<td>NAT2'6A</td>
<td>C^{262}T, G^{569}A</td>
<td>Arg^{187}Gln</td>
<td>Slow-acetylation</td>
</tr>
<tr>
<td>GSTM1' A</td>
<td>None</td>
<td>None</td>
<td>Wild-type</td>
</tr>
<tr>
<td>GSTM1' B</td>
<td>C^{543}G, exon 7</td>
<td>Lys^{172}Asn</td>
<td>No evidence of enzyme activity alteration</td>
</tr>
<tr>
<td>GSTM1'0</td>
<td>Gene deletion, intron 6</td>
<td></td>
<td>Lack of active enzyme</td>
</tr>
<tr>
<td>GSTT1' (+)</td>
<td>None</td>
<td></td>
<td>Wild-type</td>
</tr>
<tr>
<td>GSTT1'0</td>
<td>Gene deletion</td>
<td></td>
<td>Lack of active enzyme</td>
</tr>
</tbody>
</table>

Table 2. Ethnic variations of GST and NAT genetic polymorphism

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Caucasians</th>
<th>Asians</th>
<th>African-American</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAT1-rapid acetylators (NAT1'10 homozygotes)</td>
<td>0.15-0.25 [37]</td>
<td>0.21-0.26 [26, 58, 60]</td>
<td></td>
</tr>
<tr>
<td>NAT2-slow acetylators (NAT2'5B, NAT2'6A carriers)</td>
<td>0.50-0.59 [9, 21, 24]</td>
<td>0.24-0.26 [33, 50, 60]</td>
<td>0.41 [3, 22]</td>
</tr>
<tr>
<td>GSTM1 null (GSTM1'0 homozygotes)</td>
<td>0.46-0.54 [43, 54]</td>
<td>0.44-0.66 [27, 44]</td>
<td>0.16 [41]</td>
</tr>
<tr>
<td>GSTT1 null (GSTT1'0 homozygotes)</td>
<td>0.12-0.44 [29, 61]</td>
<td>0.39-0.62 [53, 59]</td>
<td>0.22 [29]</td>
</tr>
</tbody>
</table>

exhibits GSTM1 null genotype, whereas GSTT1 null homozygotes are observed in 12-44% of the general population of Europe (Tab. 2).

N-acetyltransferase and glutathione S-transferase isozymes

N-acetyltransferases are engaged in the catalysis of acetylation of many nitro- and nitroso-compounds. It is known that carcinogenic arylamine metabolites are formed as a result of bioactivation (N-oxidation) by cytochrome P450 1A2 found in the liver, while N-acetyltransferases, (mainly NAT2) competing with the cytochrome, catalyse the reaction of their detoxification through O-acetylation, which requires acetyl-CoA as a cofactor. However, arylamine metabolites, transported to the epithelium of the urinary tract, are then bioactivated through intramolecular N,O-acetyltansfer (mainly NAT1) to form highly reactive metabolites, which might react with DNA or be detoxified, e.g. by GST [57].

Glutathione S-transferase isozymes are characterised by a wide substrate specificity, which makes GST one of the main agents in the mechanism protecting from carcinogenic electrophiles and the products of oxidative stress. GSTT1 isoform catalyses the reactions of conjugation of aliphatic epoxides and halides with the reduced glutathione, whereas GSTM1 and GSTP1 isoforms metabolise benzo[a]pyrene epoxides produced as a result of bioactivation of polycyclic aromatic hydrocarbons [7, 16, 23]. People with weakened expression of different GST isozymes due to defective alleles (complete or partial deletion – GSTM1, GSTM3, GSTT1 or mutation – GSTP1) are thought to be more susceptible to the attack of carcinogens. However, aromatic and heterocyclic amines, also carcinogenic compounds, are metabolised primarily by NAT. Two basic biochemical mechanisms have been proposed to explain the relationship between N-acetylation reaction and cancer. Aromatic
amines, procarcinogens, may undergo N-acetylation, which is competitive to N-hydroxylation catalysed by cytochrome P450 dependent monooxygenases (CYP). Thus, the reactive hydroxylamines may further react with DNA, causing somatic mutations. Therefore it should be expected that rapid acetylator phenotype (NAT2*4 carriers) may protect from the induction of cancer by aromatic amines. NAT may then, unfortunately, catalyse O-acetylation of hydroxamines and contribute to the formation of acetoxy derivatives, very reactive compounds which react spontaneously with DNA forming adducts. It is therefore very important to investigate the role of NAT1 and NAT2 in the development of neoplastic diseases, including the urinary bladder cancer [15, 19, 20]. Indeed, NAT1 and NAT2 genetic polymorphisms are considered to be important factors in modifying the development of these diseases, since the expression of both NAT1 and NAT2 mRNA has been observed also in urinary bladder and urothelial cells [31, 56]. Cytosolic GSTM1 and GSTT1 forms are expressed mainly in the liver [36, 48], whereas their activity in the lungs is very low. Increased amounts of GSTP1 have also been found in intraepithelial neoplasia samples [40]. A similar high expression of GSTP1 and GSTM1 in individuals with transitional cell bladder cancer, as well as the increased level of GSTP1 in plasma of bladder cancer patients may then indicate that these enzymes are involved in the neoplasia process modification [4, 5].

NATs and GSTs as susceptibility genes in cancer of genitourinary organs

Most studies suggest a stronger link between urinary bladder cancer and genes associated with slow acetylator phenotype than with those associated with rapid acetylator phenotype [14]. A study in which the data concerning the distribution of individuals with different N-acetylation status in Europe were collected, demonstrate that the relationship between bladder cancer and NAT2 genotypes associated with slow acetylation was stronger than that with wild-type NAT2, especially in the case of smokers (odds ratio – OR: 1.5; 95% confidence interval – CI: 1.1–1.9). Slow acetylator NAT2 genotype seems therefore to be associated with moderate risk of urinary bladder cancer [38, 39]. Interestingly, that the risk of this type of cancer in slow-acetylator individuals increased by 2.5 times, if only men were included in the epidemiological analysis. In the case of German male patients with diagnosed urinary bladder cancer, a higher risk of cancer was found, related to defective GSTM1 null and NAT2*5B/6A genotype (OR: 4.39; 95% CI: 1.98–9.4), whereas in women with the same genotype combination, OR was 0.21 (95% CI: 0.06–0.80) [47]. Individuals of Italian origin showed a statistically significant relationship between slow acetylator polymorphic NAT2 genotype and the development of bladder cancer (OR: 1.72; 95% CI: 1.03–2.87). Moreover, slow acetylator genotype was associated with increased number of DNA adducts in white blood cells [42].

It is also thought that people with \(NATI^{*}10\) gene exhibit higher efficiency of N-acetylation reaction, and consequently they are rapid acetylator phenotype individuals. It is suggested that \(NATI^{*}10\) allele brings about an increase of catalytic activity of NAT1. The nucleotide substitution in \(NATI^{*}10\) is found not in the codon region, but in 3' region that does not undergo translation, and thus may change the signal of polyadenylation, and contribute to the decreased mRNA stability [19]. \(NATI^{*}10\) genetic variation, which determines rapid acetylator phenotype prevailed significantly in Japanese patients with prostate cancer, whose risk of cancer was estimated at 2.4 (95% CI: 1.0–5.6) [13]. Similarly, the frequency of \(NATI^{*}10\) alleles was found to be higher in Japanese patients suffering from urothelial transitional cell carcinoma (73.2%) than in the reference group (62.3%), although the difference was not significant [26]. However, it should be mentioned that the distribution of \(NATI^{*}10\) alleles in the Japanese population (53%) differs significantly from their distribution in the Caucasian populations (16%) [58].

The examination of the interactions between polymorphic NAT1 and NAT2 indicated much higher risk of urothelial transitional cell carcinoma in Japanese people with \(NATI^{*}10\) variant allele, associated with higher activity of NAT1 and slow acetylator NAT2 genotype (OR: 7.3; 95% CI: 2.13–32.06) than in patients with wild-type \(NATI^{*}4\) and rapid acetylator NAT2 genotype [26]. However, in the group of patients with bladder cancer, occupationally exposed to benzidine, the risk of cancer in individuals with mutated NAT2 alleles was not found to be increased (OR: 0.5; 95% CI: 0.1–1.8), which suggests the protective role of these alleles [18]. Also in the population of Danish and Swedish patients suffering from prostate cancer no statistically significant differences in the distribution of slow acetylator NAT2 genotype were observed [55].

A number of studies of GST genetic polymorphism indicate a protective role of wild-type GST genotypes in the development of cancer of the urinary organs [32]. The UK study group with prostate cancer showed a non-significant decrease in the frequency of \(GSTP1^{*}A/A\) (Ile105Ile) genotypes if compared to controls (OR: 0.4; 95% CI: 0.02–3.30). Moreover, \(GSTP1^{*}B/B\) (Val105Val) genotype appeared to be strongly associated with bladder (OR: 3.6; 95% CI: 1.4–9.2) and testicular (OR: 3.3; 95% CI: 1.5–7.7) cancers, because of high prevalence of patients homozygous for \(GSTP1^{*}B\) allele [17]. The German studies on patients with bladder cancer demonstrated a higher frequency of \(GSTM1^{*}1/1\) and \(GSTM3^{*}A/A\) genotypes than in the control group (12.32% vs. 35.43% and 83.01% vs. 67.81%, respectively). On the other hand, in the heterozygous patients, a statistically significant risk of bladder cancer
was found for both genotypes: OR: 2.31 (95% CI: 1.79–2.82) for \(\text{GSTM}^3\text{A}/\text{B}\) and 3.54 (95% CI: 2.99–4.11) for \(\text{GSTM}^1\text{A}/\text{I}\) [46]. An increased risk of urinary bladder cancer was found in another German population, but only in patients with \(\text{GSTM}1\text{null}\) genotype (OR: 1.76, 95% CI: 1.08–2.88). However \(\text{GSTT}1\text{null}\) genotype was also associated with high risk of prostate cancer (OR: 2.31; 95% CI: 1.17–4.59) [52] and almost 1.9-fold increased of the risk of bladder cancer in a Slovak population. The estimated risk of cancer in \(\text{GSTM}1\text{null}\) homozygotes increased significantly when the smoking history was taken into account (OR: 2.44, 95% CI: 1.1–5.3) [45]. Similarly, smoking in combination with \(\text{GSTT}1\text{null}\) genotype was associated with increased risk of prostate cancer in the US patients (OR: 2.31; 95% CI: 1.14–2.88), although no such relationship could be found in the case of defective \(\text{GSTM}1\text{null}\) genotype [28]. Also in the Korean population an association was observed between homozygous deletion of \(\text{GSTM}1\) gene and the risk of urinary bladder cancer (OR: 1.81; 95% CI: 1.12–2.93), whereas in individuals with double copies of defective \(\text{GSTT}1\) gene there were no statistically significant differences in the distribution between the two groups examined [30]. In a study on a German population no significant differences were observed in the frequency of \(\text{GSTM}1\text{null}\) and \(\text{GSTT}1\text{null}\) genotypes between the group of bladder cancer patients (59.2% and 17.8%, respectively) and the group of healthy people (57.8% and 21.5%, respectively) [47]. A study carried out in the UK on a Caucasian population also demonstrated insignificant differences between the group of patients with bladder cancer and the controls. The frequency of \(\text{GSTM}1\text{null}\) genotype was found to be 42% for the control group and 40% for the group of patients [62]. Lin et al [35] undertook a study to estimate the risk of urinary bladder cancer in different ethnic groups of patients in Los Angeles. The OR value was similar for the different groups and equalled 1.4 (95% CI: 0.94–2.1). The differences in the frequency of \(\text{GSTM}1\) genotype in the study and control groups were not statistically significant. Association between GST, NAT genetic polymorphism and the occurrence of bladder and prostate cancers is summarised in Table 3.

Concluding remarks

Not only the human population is genetically differentiated, but also individual susceptibility to carcinogens present in the environment is difficult to define. Therefore, the results of epidemiological studies of metabolic polymorphisms in people suffering from neoplasm of urinary organs are not explicit. Some researchers report the relationship between certain genetic variations of NAT or GST, but others fail to prove it.

A single risk factor of bladder cancer, such as genetic polymorphism of one of the numerous xenobiotic metabolising enzymes, does not seem to provide a full explanation of the cause of the disease. Knowing that a number of enzymes are engaged in the transformation of toxic chemicals, several genetic polymorphisms: NAT1, NAT2, \(\text{GSTM}1\) and \(\text{GSTT}1\) have to be considered when investigating the etiology of the urinary bladder cancer [1, 23].

The analysis of the association between metabolic genotype and common diseases is still at a critical point. The effect of interactions between the genes and between the environment and the genes on the development of cancer seems to be substantial, but it is necessary to identify their total contribution by exploring many risk factors and performing extensive genotyping studies among numerous normals and cases. It is also very important to identify genes with high prevalence of alleles conferring a low increase or
decrease in cancer risk. The tools that would help this strategy have been developing rapidly due to high throughput technologies, including genotyping techniques relying on a base extension (minisequencing) and hybridisation of allele-specific probes (microarrays, TaqMan assays). The availability of automation in genetic polymorphism analysis creates a possibility to get more information about various population-specific allelic frequencies [8]. However, the knowledge about the cancer risk of urinary organs, based on molecular epidemiological studies seems to be insufficient to find out the etiology of a given disease. Another challenge in cancer genetics to be overcome is a functional characterization of nucleotide polymorphism of xenobiotic metabolizing enzymes. The loss of expression or the decreased enzymatic activity has been explained by transcriptional or translational repression. In prostate cancer cells, there was found cytosine methylation in GSTP1 regulatory sequences associated with the reduction of GSTP1 gene expression [51]. Moreover, reduced or compromised translation, protein folding, degradation or other mechanisms may indicate NAT1 and NAT2 allelic variants that encode slow or fast acetylator NAT phenotypes [10, 11].

Although genetic polymorphisms of biotransformation enzymes might be a critical point, it is clear, that the complex, multiphase process of carcinogenesis is controlled by many factors. One of them is the genetic predisposition, related to the presence of genetic polymorphisms of the enzymes involved in the activation and conjugation of potentially genotoxic compounds. The understanding of the relationship between biochemical markers (anti- and prooxidants, microelements), molecular markers (expression of oncoproteins), environmental and occupational exposure and individual genetic NAT and GST polymorphism will expand the knowledge of the etiology of neoplastic diseases of urinary organs.

References


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