Risks on N-acetyltransferase 2 and bladder cancer: a meta-analysis

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Introduction
The morbidity rate and mortality rate of bladder tumor rank as the first one in the urinary system tumors, and tend to increase year by year. Clinically, bladder tumor is divided into superficial bladder cancer and invasive bladder cancer. At present, the main treatment of the bladder cancer is operation and supplemented by radiotherapy and chemotherapy. However, the recurrence rate is relatively high and 5-year survival rate is approximately 50%.¹⁻⁴ How to find a more effective biomedical treatment to reduce the recurrence rate and metastatic rate of bladder cancer has become a critical problem in the world?

The risk of bladder cancer relates to NAT2 slow acetylation was confirmed by domestic and foreign scholars.⁵⁻¹¹ However, this relationship was not approved in some researches. Part of the reasons can be attributed to the differences in statistical sample size. Only a small amount of research shows significant difference. The purpose of this meta-analysis explores the risk of NAT2 slow acetylation and the bladder cancer.
**Materials and methods**

**Retrieval of articles**

By retrieving electronic database, like PubMed, Cochran, McGrane English databases, CBM, CNKI, and other databases, the author tested the status of NAT2 that was taken from patients with bladder cancer and control group. At the same time, also using reference articles and publications for data retrieval that were published from January 1980 to February 2014. The English key words include NAT2 or N-acetyl and bladder cancer. Using the combined form of key words and free words, all of the above searching strategies are obtained from the pre-searching methods. Three doctors operated the article retrieval independently. In order to reduce the leakage of literature, we refined search for reference documentation that were brought into literature.

**Inclusion criteria and exclusion criteria**

The following are inclusion criteria. 1) English literature; 2) randomized controlled trial, prospective or retrospective control study, and cohort study; 3) data should be complete and creditable; and 4) accord with retrieval condition and requirement.

The following are exclusion criteria. 1) Language is non-English literature; 2) no summary; 3) no specific statistical data of NAT2; and 4) no control group treatment, a review of the literature, comments, and in vitro study of tumor.

**Statistical analysis**

By comparing these specific values and their corresponding 95% confidence interval (CI) with the number of research object in research, the risk of being infected with bladder cancer was found associate with slow NAT2 acetylation by some researches, and check whether there are any corresponding sample bias. At the same time, considering the variable within and between the researches according to the previous studies.\footnote{12–15} Data were analyzed using STATA Version 6.0 software (StataCorp LP, College Station, TX, USA), using the META module.

**Results**

Figure 1 shows the flow chart of study selection. Through the screening of the database, 20 studies that analyzing the risks of being infected by NAT2 and bladder cancer in the controlled study were brought into analysis (Figure 2 and Table 1).\footnote{7–26} including 2,463 patients with bladder cancer cases and 3,451 cases of control group which are all without randomness.

Excluding the NAT2 phenotype and to prevent bias, only patients group and the control group were considered for subgroup analysis research. There were account 12 articles cited by phenotypic description of NAT2 gene and account eight by genotype. However, according to the aim of this meta-analysis, the present only cited the studies that described the genetic data. In some researches, healthy people are treated as controlled group, and in some of the researches, hospitalized patients with malignant tumor are concerned. The age of control group is matched up with cases group. Smoking history and the patient’s occupational exposure in some studies are analyzed.

**Relationship between feature genes of NAT2 and bladder cancer**

We made a forest plot for 3,451 cases and 3,451 control groups (Figure 2). Figure 2 shows 20 studies that include forest odds ratio analysis and 95% CI. This suggests the possibility of the man with changeable NAT2 gene become a bladder cancer patient. In the overall sample, the slow acetylator’s odds ratio is 1.31 (95% CI: 1.11–1.55). The heterogeneity was studied by statistical analysis ($Q=35.6$, $df=21$, $P=0.024$). There is some evidence that may be due in part of the differences in the allocation method of the state of NAT2.\footnote{27–31} By layer approaching, the study focuses on phenotypic, the obtained odds ratio is 1.34 (95% CI: 1.08–1.69; test for heterogeneity: $Q=17.24$, $df=12$, $P=0.14$). Genotyping studies and pooled odds ratio are 1.27 (95% CI: 0.97–1.67; test for heterogeneity $Q=18.03$, $df=8$, $P=0.021$).

The result was drawn from the analysis of pooled data of research based on many factors. The risk of the bladder cancer that defined the phenotype and genotype of NAT2...
on slow acetylation in patients with bladder cancer cases had exposed to more carcinogens or the smokers.

**Publication bias**

Publication bias was investigated by Begg’s funnel plot, and the funnel plot’s asymmetry was further assessed by Egger’s test. As shown in Figure 4, there was low possibility of asymmetry in the dominant model of this meta-analysis. Besides, finding from the Egger’s test further suggested that there was no significant risk of publication bias ($P=0.183$).

**Discussion**

In the late 1970s and early 1980s, many experts started to study the NAT2 slow acetylator status which is a risk factor for bladder cancer, and is put forward by many experts and scholars. This meta-analysis aimed to clarify whether NAT2 slow acetylator status can increase the risk of bladder cancer.

The bladder cancer is one of the few cancers that have been found to be involved directly with environmental carcinogens. Exposure to industrial chemicals, aromatic amines, is one of
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the environmental factors that can cause bladder cancer. People who engage in rubber, dyestuff, and printing industry will be more easily affected by chemical composition. In addition, the factors of inhalation of diesel exhaust and smoke are also included. These factories bring aromatic amine that mainly include high polymer aromatic amines, such as naphthylamine, 4-aminobiphenyl, benzidine, and their N-hydroxylated derivative. All of these are known or potential NAT2 substrates.36,37 Currently, the view, exposure to carcinogens is a risk factor for bladder cancer, has been a broad consensus. That is the reason why NAT2 slow acetylator has been recognized as a risk factor for assessing bladder cancer risk. At present, some studies failed to prove the relationship between NAT2 slow acetylator status and bladder cancer, and the main reason can be attributed to the differences in statistical sample size. Only a few studies in the bilateral 5% statistical level have significant sexual differences. In our study, we also cannot discover the significant sexual differences (data not shown). The advantage of this study is that the statistical data can be combined and analyzed by different methods.

Influence factors of the disease, we will follow contrast principles of single variable and adopt the allele frequency research, including racial and geographic origin. However, in some previous studies, considering race and other reasons, we do the mixed study of contrasted race and geographical origin. Thus, unmatched cases and contrast are the sources of bias. In addition, as previously mentioned, in view of any polymorphism, carcinogen metabolism enzyme is impossible to increase the risk of bladder cancer twice or 1.5 times than the associated smaller risk, there are many biases of NAT2 status in most published studies.25,38,39 Many published reports are based on the comparison of cases and

| Table 1 Comparative study of NAT2 and risk of bladder cancer patients |
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| Authors          | Country        | Analysis method | Bladder cancer cases (n) | Acetylation (%) | Control group (n) | Acetylation (%) | Exposure factor |
| Lower et al31    | Sweden and Denmark | SMZ phenotype     | 186              | 65              | 192              | 60             | Smoking history |
| Cartwright et al9 | UK             | DDS phenotype     | 111              | 67              | 207              | 57             | Smoking history |
| Woodhouse et al28 | UK             | INH phenotype     | 30               | 70              | 27               | 59             | Occupation history |
| Miller and Cosgriff24 | USA    | SMZ phenotype     | 26               | 46              | 26               | 69             | Smoking history |
| Evans et al9     | UK             | SMZ phenotype     | 100              | 66              | 852              | 60             | Occupation history |
| Ladero et al11   | Spain          | SMZ phenotype     | 130              | 64              | 157              | 57             | Smoking history |
| Hanssen et al16  | Germany        | SMZ phenotype     | 105              | 62              | 42               | 43             | Occupation history |
| Mommsen et al37  | Denmark        | SMZ phenotype     | 228              | 64              | 100              | 54             | Smoking history |
| Karakaya et al20  | Turkey         | SMZ phenotype     | 23               | 39              | 109              | 62             | Occupation history |
| Kaisary et al27   | UK             | DDS phenotype     | 98               | 60              | 110              | 49             | Occupation history |
| Horai et al24    | Japan          | DDS phenotype     | 51               | 6               | 202              | 6              | Occupation history |
| Hanke and Krajewska14 | Poland   | INH phenotype     | 67               | 70              | 22               | 45             | Occupation history |
| Hayes et al17    | People’s Republic of China | DDS phenotype | 38            | 9               | 43               | 23             | Occupation history |
| Risch et al11    | UK             | NAT2 genotype     | 189              | 67              | 59               | 44             | Occupation history |
| Brockmüller et al4  | Germany      | NAT2 genotype     | 374              | 62              | 373              | 58             | Occupation history |
| Oikkels et al37   | Denmark        | NAT2 genotype     | 254              | 61              | 242              | 56             | Occupation history |
| Miller et al24    | USA            | NAT2 genotype     | 230              | 37              | 203              | 48             | Occupation history |
| Schnakenberg et al39 | Germany   | NAT2 genotype     | 60               | 70              | 154              | 61             | Occupation history |
| Filadis et al10   | Greece         | NAT2 genotype     | 89               | 58              | 147              | 38             | Occupation history |
| Hsieh et al20     | Taiwan         | NAT2 genotype     | 74               | 21              | 184              | 24             | Occupation history |

Abbreviations: DDS, Denys–Drash syndrome; INH, isoniazid; NAT2, N-acetyltransferase 2; SMZ, sulfamethazine.
control groups. By adopting this, comparison can show the susceptibility to NAT2 slow acetylator status. By this meta-analysis, we can get that although heterogenicity exists in the relationship between NAT2 slow acetylator status and bladder cancer, slow acetylator status still can increase the risk of bladder cancer. Because of gene frequency of occurrence in population, area and other factors are pretty high. It means that the status of NAT2 will increase the morbidity of bladder cancer obviously. Although NAT2 is present in the bladder epithelium and its expression is not high, the status of fast acetylation will not increase the morbidity of bladder cancer. If diversity of genetic susceptibility exists in bladder cancer, then maybe the correspondent gene positions are not the same. However, the expressive of some genotypic combination are higher. For example, NAT2 slow acetylation status combined Glutathione S-transferase M1 defects. The possibility of such inconclusive studies have been checked so far because of a shortage of convincing evidence. Maybe future work will be influenced by the decrease of match detection. By adding sampling rate and specific groups to prove gene, polymorphism and environmental factor will have an influence on morbidity of bladder cancer.

In this study, we analyzed the correlation between NAT2 slow acetylation and the risk of bladder cancer. The result showed that the individual differences of bladder cancer susceptibility might be part of the metabolism of carcinogens. Slow acetylation status of bladder cancer is associated with the bladder cancer risks.

In conclusion, the status of NAT2 slow N-acetylation is associated with bladder cancer risks, and may increase the risk of bladder cancer.

Disclosure
The authors report no conflicts of interest in this work.

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