REVIEW

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

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Background: It is known that bladder cancer disease is closely related to aromatic amine compounds, which could cause cancer by regulating of N-acetylation and N-acetyltransferase 1 and 2 (NAT1 and NAT2). The NAT2 slowed acetylation and would increase the risk of bladder cancer, with tobacco smoke being regarded as a risk factor for this increased risk. However, the relationship between NAT2 slow acetylation and bladder cancer is still debatable at present. This study aims to explore preliminarily correlation of NAT2 slow acetylation and the risk of bladder cancer.

Methods: The articles were searched from PubMed, Cochran, McGrane English databases, CBM, CNKI, and other databases. The extraction of bladder cancer patients and a control group related with the *NAT2* gene were detected by the state, and the referenced articles and publications were also used for data retrieval. Using a random effects model, the model assumes that the studies included in the analysis cases belong to the overall population in the study of random sampling, and considering the variables within and between studies. Data were analyzed using STATA Version 6.0 software, using the META module. According to the inclusion and exclusion criteria of the literature study, 20 independent studies are included in this meta-analysis.

Results: The results showed that the individual differences of bladder cancer susceptibility might be part of the metabolism of carcinogens. Slow acetylation status of bladder cancer associated with the pooled odds ratio was 1.31 (95% confidence interval: 1.11–1.55).

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

Keywords: N-acetyltransferase 2, NAT2 slow acetylation status, bladder cancer, 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.[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),[7–26] including 2,463 patients with bladder cancer cases and 3,451 cases of control group which are all without randomness.
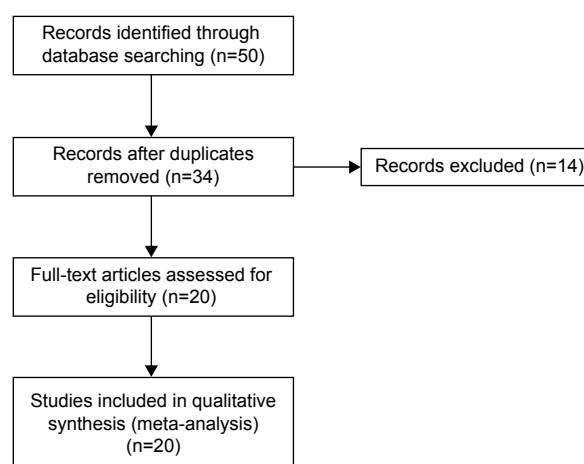

Figure 1 Flowchart of study selection.

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.[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
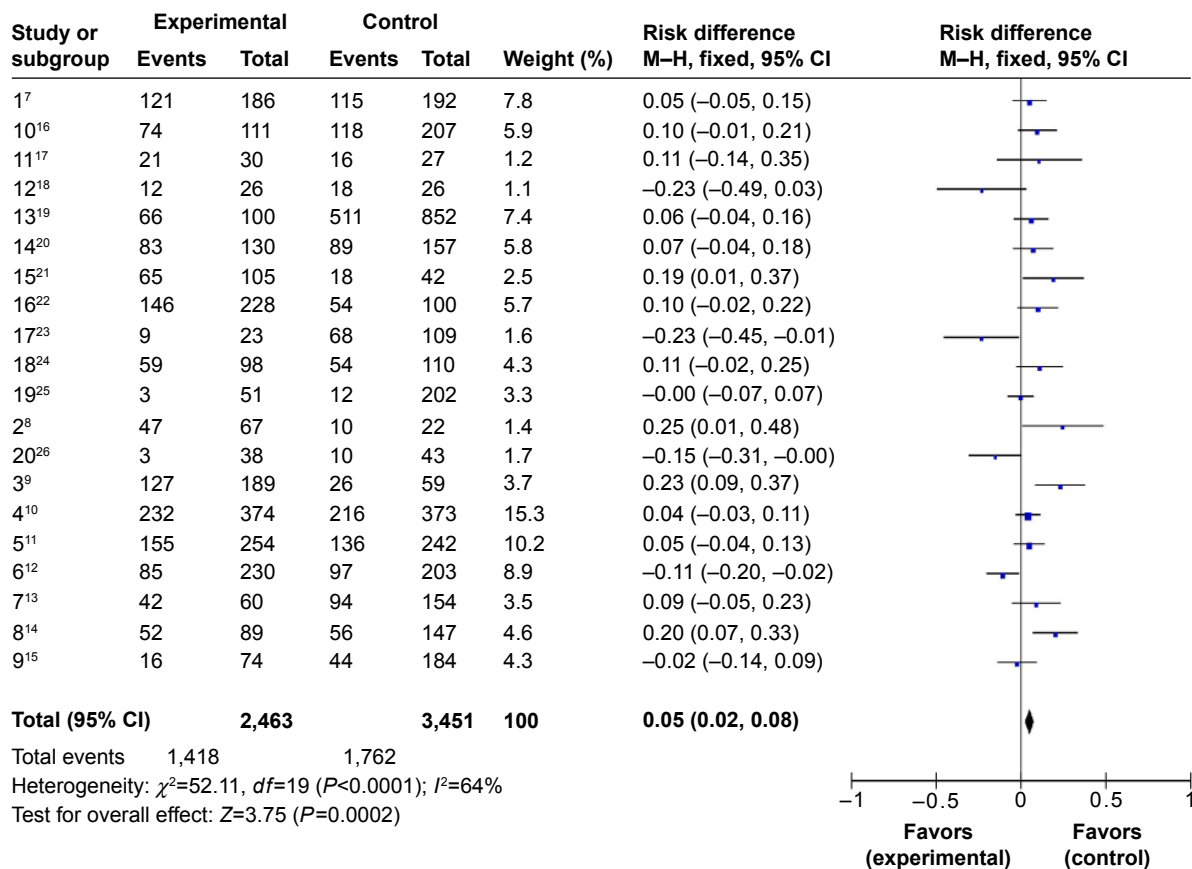
Study or subgroup	Experimental		Control		Weight (%)	Risk difference M–H, fixed, 95% CI	Risk difference M–H, fixed, 95% CI
	Events	Total	Events	Total			
1[7]	121	186	115	192	7.8	0.05 (–0.05, 0.15)	
10[16]	74	111	118	207	5.9	0.10 (–0.01, 0.21)	
11[17]	21	30	16	27	1.2	0.11 (–0.14, 0.35)	
12[18]	12	26	18	26	1.1	–0.23 (–0.49, 0.03)	
13[19]	66	100	511	852	7.4	0.06 (–0.04, 0.16)	
14[20]	83	130	89	157	5.8	0.07 (–0.04, 0.18)	
15[21]	65	105	18	42	2.5	0.19 (0.01, 0.37)	
16[22]	146	228	54	100	5.7	0.10 (–0.02, 0.22)	
17[23]	9	23	68	109	1.6	–0.23 (–0.45, –0.01)	
18[24]	59	98	54	110	4.3	0.11 (–0.02, 0.25)	
19[25]	3	51	12	202	3.3	–0.00 (–0.07, 0.07)	
2[8]	47	67	10	22	1.4	0.25 (0.01, 0.48)	
20[26]	3	38	10	43	1.7	–0.15 (–0.31, –0.00)	
3[9]	127	189	26	59	3.7	0.23 (0.09, 0.37)	
4[10]	232	374	216	373	15.3	0.04 (–0.03, 0.11)	
5[11]	155	254	136	242	10.2	0.05 (–0.04, 0.13)	
6[12]	85	230	97	203	8.9	–0.11 (–0.20, –0.02)	
7[13]	42	60	94	154	3.5	0.09 (–0.05, 0.23)	
8[14]	52	89	56	147	4.6	0.20 (0.07, 0.33)	
9[15]	16	74	44	184	4.3	–0.02 (–0.14, 0.09)	
Total (95% CI)		**2,463**		**3,451**	**100**	**0.05 (0.02, 0.08)**	
Total events	1,418		1,762				

Heterogeneity: χ^2=52.11, df=19 (P<0.0001); I^2=64%
Test for overall effect: Z=3.75 (P=0.0002)

Figure 2 Forest plot for the risk of bladder cancer influenced by NAT2.
Abbreviations: CI, confidence interval; M–H, Mantel–Haenszel.

state is 1:3 (95% CI: 1.18–1.64; heterogeneity inspection: Q=28.09, df=17, P=0.04).[32,33] Only in the study of pooled phenotype, the odds ratio is 1.36 (95% CI: 1.08–1.70; heterogeneity inspection: Q=16.86, df=11, P=0.12), and only in the study of aggregate genotype, the odds ratio is 1.44 (95% CI: 1.10–1.89; heterogeneity inspection: Q=11.21, df=5, P=0.05). It is concluded from three Asian studies (Figure 3A) that the ratio is 0.75 (95% CI: 0.45–1.28).[33–35]

Relationship between slow acetylation and bladder cancer

In this research, we investigate and summarize from smoking (Figure 3A) or the influence of occupational exposure (Figure 3B). Odds ratio of smoking group is 1.21 (95% CI: 0.826–0.827; heterogeneity inspection: Q=0.6435, P=0.842; the figure of area under the curve is 0.6881). Odds ratio of occupational exposure group is 1.9 (95% CI: 0.589–0.656; heterogeneity inspection: Q=0.6433, P=0.623; the figure of area under the curve is 0.6879). Table 1 shows the risk research on patients with slow acetylation and bladder cancer, and the interaction between these risks.[23] Excess

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.[10–14] 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

Table 1 Comparative study of NAT2 and risk of bladder cancer patients

Authors	Country	Analysis method	Bladder cancer cases (n)	Acetylation (%)	Control group (n)	Acetylation (%)	Exposure factor
Lower et al[32]	Sweden and Denmark	SMZ phenotype	186	65	192	60	Smoking history
Cartwright et al[6]	UK	DDS phenotype	111	67	207	57	Smoking history Occupation history
Woodhouse et al[28]	UK	INH phenotype	30	70	27	59	
Miller and Cosgriff[34]	USA	SMZ phenotype	26	46	26	69	Smoking history Occupation history
Evans et al[9]	UK	SMZ phenotype	100	66	852	60	Smoking history Occupation history
Ladero et al[31]	Spain	SMZ phenotype	130	64	157	57	Smoking history Occupation history
Hanssen et al[16]	Germany	SMZ phenotype	105	62	42	43	Smoking history Occupation history
Mommsen et al[29]	Denmark	SMZ phenotype	228	64	100	54	
Karakaya et al[30]	Turkey	SMZ phenotype	23	39	109	62	
Kaisary et al[27]	UK	DDS phenotype	98	60	110	49	Smoking history Occupation history
Horai et al[24]	Japan	DDS phenotype	51	6	202	6	Smoking history Occupation history
Hanke and Krajewska[14]	Poland	INH phenotype	67	70	22	45	Occupation history
Hayes et al[17]	People's Republic of China	DDS phenotype	38	9	43	23	Smoking history Occupation history
Risch et al[11]	UK	NAT2 genotype	189	67	59	44	Smoking history Occupation history
Brockmöller et al[4]	Germany	NAT2 genotype	374	62	373	58	Smoking history Occupation history
Okkels et al[37]	Denmark	NAT2 genotype	254	61	242	56	Smoking history Occupation history
Miller et al[34]	USA	NAT2 genotype	230	37	203	48	Smoking history Occupation history
Schnakenberg et al[39]	Germany	NAT2 genotype	60	70	154	61	Smoking history Occupation history
Filiadis et al[10]	Greece	NAT2 genotype	89	58	147	38	Smoking history
Hsieh et al[26]	Taiwan	NAT2 genotype	74	21	184	24	Smoking history

Abbreviations: DDS, Denys–Drash syndrome; INH, isoniazid; NAT2, N-acetyltransferase 2; SMZ, sulfamethazine.

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
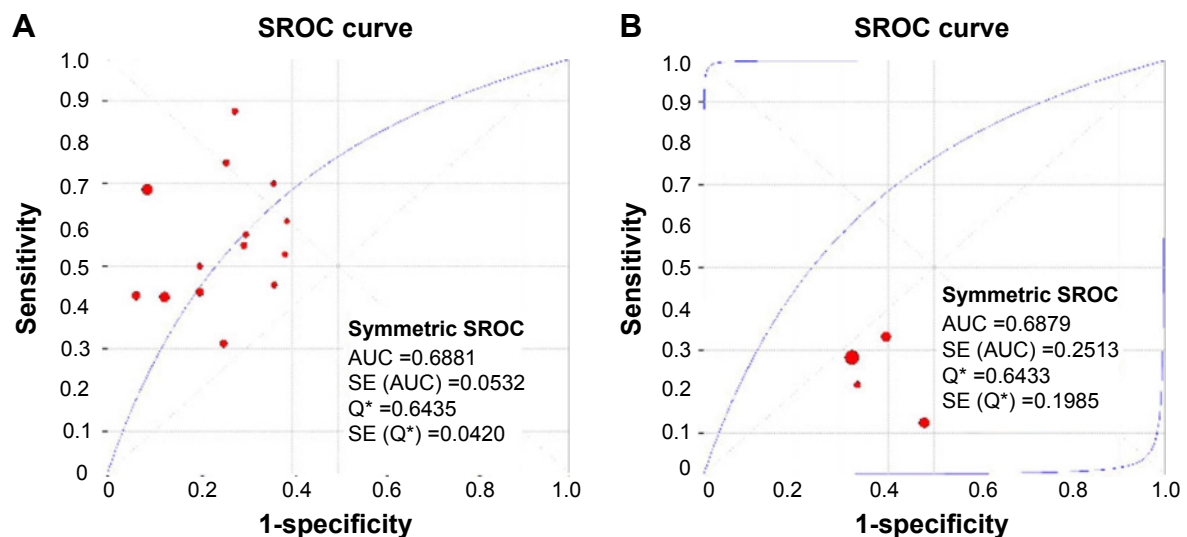
A SROC curve

Symmetric SROC
AUC =0.6881
SE (AUC) =0.0532
Q* =0.6435
SE (Q*) =0.0420

B SROC curve

Symmetric SROC
AUC =0.6879
SE (AUC) =0.2513
Q* =0.6433
SE (Q*) =0.1985

Figure 3 The relationship between smoking and occupational exposure and bladder cancer.
Notes: (**A**) The relationship between smoking and bladder cancer. (**B**) The relationship between occupational exposure and bladder cancer.
Abbreviations: AUC, area under the curve; SE, standard error; SROC, summary receiver operating characteristics; Q*, Q-statistics.

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.[40] 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.[40,41] 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.

Figure 4 Funnel plot for the detection of the publication bias in this meta-analysis.
Abbreviation: SE, standard error.

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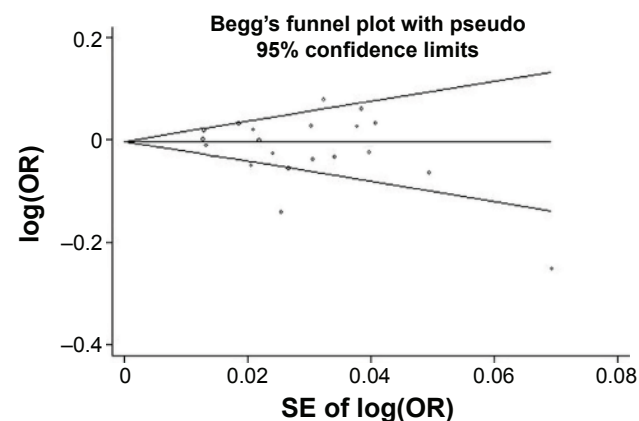
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