Meta-analysis of the relationship between slow acetylation of N-acetyl transferase 2 and the risk of bladder cancer

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ABSTRACT. The incidence of bladder cancer is closely associated with exposure to aromatic amines, that can cause cancer only after metabolic activation regulated by N-acetyl transferase 1 and 2 (NAT1 and NAT2). Many studies have indicated that slow acetylation of NAT2 increases the risk of bladder cancer. The major risk factor is tobacco smoke; however, some studies have failed to prove this. This study attempted to explore the correlation between NAT2 slow acetylation and bladder cancer risk through a meta-analysis of published case-control studies. Studies detecting *NAT2* gene status in bladder cancer patients and healthy controls were retrieved

from PubMed, Cochrane, EMchrane, CBM, and CNKI. We retrieved the data of cited articles and publications to identify and compare NAT2 gene in bladder cancer patients and healthy controls. The variables within and between the studies were also considered. The META module in the Stata v.6.0 software was used for data analysis. Twenty independent studies were enrolled in our meta-analysis according to the inclusion and exclusion criteria. Individual differences in the bladder cancer susceptibility were, in part, attributed to the effect of carcinogens. The merged odds ratio of the effect of slow acetylation on bladder cancer was 1.31 (95% confidence interval = 1.11-1.55). In conclusion, NAT2 slow acetylation state was associated with bladder cancer risk, and was shown to modestly increase the risk of bladder cancer.

Key words: NAT2; NAT2 slow acetylation; Bladder cancer

INTRODUCTION

The bladder tumor-related morbidity and mortality rate are highest among tumors in urinary system, and the incidence of this malignant tumor has increased gradually. Bladder tumor is clinically divided into superficial bladder cancer and invasive bladder cancer. At present, surgery, accompanied by radiation and chemotherapy, is the main treatment option for bladder cancer. However, its recurrence rate remains high, while the 5-year survival rate remains low at approximately 50% (Marcus et al., 2000). Therefore, it has become imperative to discover more effective biological medical treatment strategies to reduce the rate of recurrence and metastasis of bladder cancer; however, this has proven to be a challenging task.

Several scholars have confirmed an association between bladder cancer risk and N- acetyl transferase-2 (NAT2) slow acetylation (Cartwright et al., 1982; Evans et al., 1983; DerSimonian et al., 1986; Dickersin et al., 1992; Risch et al., 1995; ONS, 1996; Filiadis et al., 1999). However, some studies have failed to prove this relationship, which may be attributed to the statistical difference in sample size. Very few research studies have exhibited significant differences at the bilateral 5% statistical level. The aim of this meta-analysis was to explore the relationship between NAT2 and bladder cancer risk.

MATERIAL AND METHODS

Literature search

Research papers analyzing the genetic status of NAT2 in bladder cancer patients and healthy controls were searched from the PubMed, Cochrane, EMchrane, CBM, and CNKI databases. The key words for this search included NAT2 or N-acetyl transferase-2, and bladder cancer. The search strategy was implemented according to the preview of retrieved literatures in the form of subject words combined with free words. The literature was surveyed independently by three doctors. The studies referred to in the enrolled articles were subjected to a secondary search, in order to reduce the leakage.

The studies were included based on conformance with the following criteria: 1) the

studies were reported in English only; 2) the literature reported a random-controlled, prospective or retrospective case-control, or cohort study; 3) the data were integrated and credible; and 4) the study conformed with the retrieval requirements.

Studies were not included based on the following exclusion criteria: 1) studies not reported in English; 2) lack of an abstract; 3) lack of NAT2-specific statistical data; and 4) the lack of a control; in addition, review literature, comments, and *in vitro* tumor studies were excluded from this meta-analysis.

Statistical analysis

A number of included studies reported an association between the risk of bladder cancer and NAT2 slow acetylation; in this analysis, the odds ratio (OR) values of these studies [corresponding to a 95% confidence interval (CI)] were compared to determine any corresponding sample size bias. In case of heterogeneity, which may be involved in the research, we adopted the random-effect model to make an assumption in this study. The META module in the Stata (v.6.0) software (Stata Corporation, College Station, TX, USA) was used for data analysis.

RESULTS

A total of 20 case-control studies analyzing NAT2 and the risk of bladder cancer were enrolled in our analysis (Fleiss, 1980; Evans et al., 1983; Hanssen et al., 1985; DerSimonian et al., 1986; Pearce et al., 1989; Horai et al., 1989; Ponder, 1991; Dickersin et al., 1992; Hayes et al., 1993; Greenland, 1994; Smith et al., 1995; Risch et al., 1995; Houlston, 1996; Stanley et al., 1996; Filiadis et al., 1999; Hirvonen, 1999; Hsieh et al., 1999; Brennan et al., 2000) (Table 1). Studies wherein the NAT2 phenotype only analyzed in a patient group or control group were ruled out to prevent bias. Twelve studies described the NAT2 gene by phenotype, and 7 described the same by genotype; only genetic data were included in our study according to the aims of our meta-analysis. The control groups were composed of either healthy people or hospitalized patients diagnosed with non-malignant tumors in the different studies. The age of the subjects in the control group was matched to that of the tumor patients in the individual studies. Some studies also analyzed the smoking history and the history of occupational exposure.

Twenty studies, including 2462 patients with bladder cancer and 3450 controls, were enrolled in this meta-analysis (Figure 1). A Forest map indicated the OR and 95%CI of each study that indicated the possibility of patients with changes in the NAT2 informative gene developing bladder cancer. The overall OR of slow acetylation was 1.31 (95%CI = 1.11-1.55); the heterogeneity between studies was statistically analyzed (Q = 35.6, d.f. = 21, P = 0.024). Some studies demonstrated this, in part, maybe due to the differences in the NAT2 determined by distinct methods (Woodhouse et al., 1982; Ladero et al., 1985; Mommsen et al., 1985; Karakaya et al., 1986; Kaisary et al., 1987). The stratification analysis revealed that the OR of the studies researching the NAT2 phenotype was 1.34 (95%CI = 1.08-1.69; heterogeneity inspection: Q = 17.24, d.f. = 12, P = 0.14), and the NAT2 genotype was 1.27 (95%CI = 0.97-1.67; heterogeneity inspection Q = 18.03, d.f. = 8, P = 0.021).

The results from pooled data were based on many factors. The OR of bladder cancer based on the NAT2 status defined by phenotype and genotype was 1.39 (95%CI = 1.18-1.64; heterogeneity inspection: Q = 28.09, d.f. = 17, P = 0.04) (Lower et al., 1979; Mesrobian et al.,

Table 1. Case-control studies investigating the correlation between NAT2 and bladder cancer risk.

Authors	Country	Analytical method	Bladder cancer cases	Acetylation (%)	Control cases	Acetylation (%)	Exposure factor
Lower et al.	Sweden, Denmark	SMZ phenotype	186	65	192	60	Smoking history
Cartwright et al.	UK	DDS phenotype	111	67	207	57	Smoking history occupational exposure history
Woodhouse et al.	UK	INH phenotype	30	70	27	59	
Miller and Cosgriff	United States	SMZ phenotype	26	46	26	69	Smoking history occupational exposure history
Evans et al.	UK	SMZ phenotype	100	66	852	60	Smoking history occupational exposure history
Ladero et al.	Spain	SMZ phenotype	130	64	157	57	Smoking history occupational exposure history
Hanssen et al.	Germany	SMZ phenotype	105	62	42	43	Smoking history occupational exposure history
Mommsen et al.	Denmark	SMZ phenotype	228	64	100	54	
Karakaya et al.	Turkey	SMZ phenotype	23	39	109	62	
Kaisary et al.	UK	DDS phenotype	98	60	110	49	Smoking history occupational exposure history
Horai et al.	Japan	DDS phenotype	51	6	202	6	Smoking history occupational exposure history
Hanke and Krajewska	Poland	INH phenotype	67	70	22	45	Occupational exposure history
Hayes et al.	China	DDS phenotype	38	9	43	23	Smoking history occupational exposure history
Risch et al.	UK	NAT2 genotype	189	67	59	44	Smoking history occupational exposure history
Brockmoller et al.	Germany	NAT2 genotype	374	62	373	58	Smoking history occupational exposure history
Okkels et al.	Denmark	NAT2 genotype	254	61	242	56	Smoking history occupational exposure history
Taylor et al.	United States	NAT2 genotype	230	37	203	48	Smoking history occupational exposure history
Schnakenberg et al.	Germany	NAT2 genotype	60	70	154	61	Smoking history occupational exposure history
Filiadis et al.	Greece	NAT2 genotype	89	58	147	38	Smoking history
Hsieh et al.	Taiwan	NAT2 genotype	74	21	184	24	Smoking history

1988). The pooled phenotypic studies showed an OR of 1.36 (95%CI = 1.08-1.70; heterogeneity inspection: Q = 16.86, d.f. = 11, P = 0.12) and the aggregate of all genotypic literature (only) showed an OR of 1.44 (95%CI = 1.10-1.89; heterogeneity inspection: Q = 11.21, d.f. = 5, P = 0.05). The Odds ratio of the three Asian studies was 0.75 (95%CI = 0:45-1.28) (Figure 2) (Miller et al., 1983; Mesrobian et al., 1988; Windmill et al., 2000).

The slow acetylation status may be associated with a certain type of bladder cancer. The NAT2 status in bladder cancer was not described according to the histological type (in detail) in a majority of the studies, which impeded the subgroup analysis. However, a number of studies demonstrated that the pathology of urothelial carcinoma patients was transitional cell carcinoma.

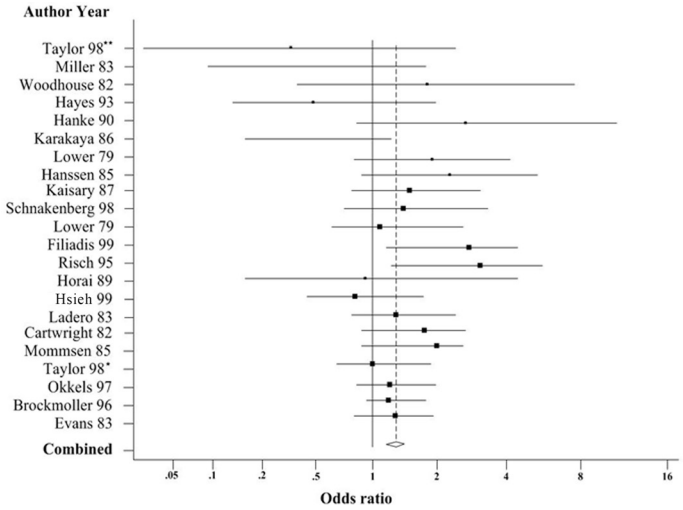

Figure 1. Forest map of NAT2 with respect to bladder cancer risk.

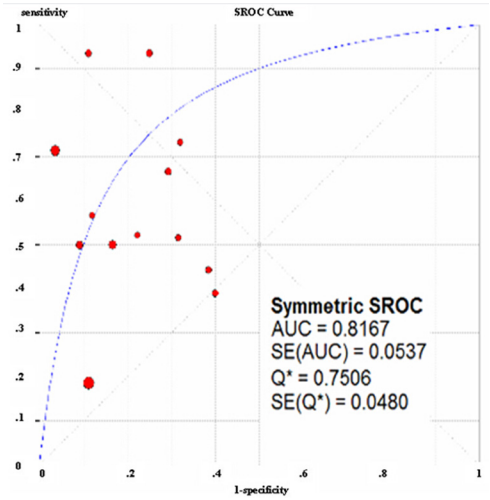

Figure 2. Odds ratio of the three Asian studies on NAT2. Pooled data of phenotype and genotype defined the NAT2 status.

We further investigated the influence of smoking or occupational exposure on bladder cancer in general (Figures 3 and 4). Table 1 summarizes the impact of slow acetylation on the risk of bladder cancer, and the interaction between these risks (Smith et al., 1995). Bladder cancer patients showing excessively slow acetylation were generally more exposed to carcinogens, or were smokers.

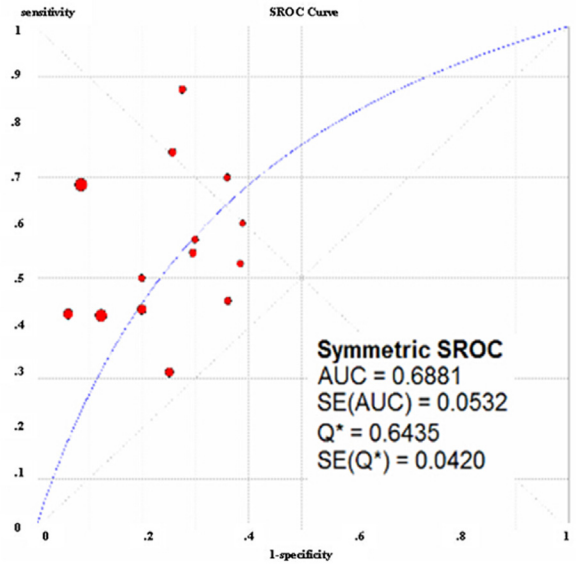

Figure 3. Correlation between smoking history and bladder cancer risk. Bladder cancer patients showing excessively slow acetylation were generally more exposed to carcinogens.

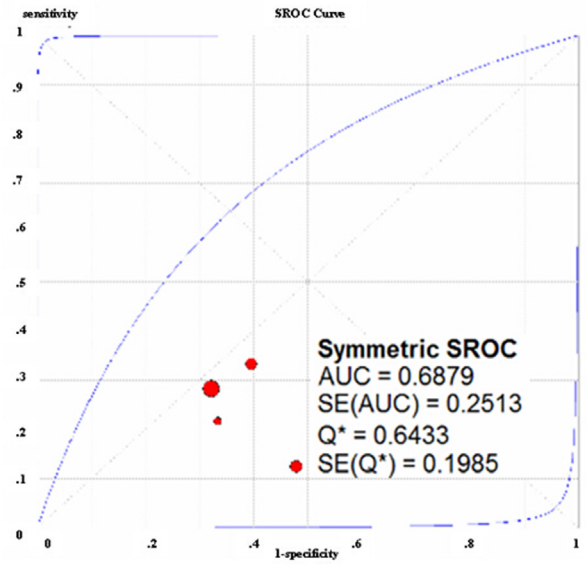

Figure 4. Correlation between occupational exposure and bladder cancer risk. Bladder cancer patients showing excessively slow acetylation were generally more exposed to smoking.

DISCUSSION

The NAT2 slow acetylation status was considered to be a risk factor for bladder cancer early in the 1970s, and a large number of studies attempted to investigate the relationship between the NAT2 status and the risk of bladder cancer (Fleiss, 1980; Hanke et al., 1990; Greenland, 1994; Risch et al., 1995; Filiadis et al., 1999). The aim of this meta-analysis was to clarify if the NAT2 status increases the risk of bladder cancer.

Bladder cancer is one of the few cancers directly implicated with the presence of environmental carcinogens. These environmental factors include industrial exposure of aromatic amines that are mainly released from rubber, dyes, printing, and with diesel engine exhaust emissions. These patients are generally exposed to high levels of aromatic amines, such as naphthylamine, 4-aminobiphenyl, benzidine, and their N-hydroxyl derivatives, which are known or potential NAT2 substrates (Okkels et al., 1997; Peluso et al., 1998; Mostafa et al., 1999). At present, exposure to carcinogens is considered, by wide consensus, to be risk factors for bladder cancer; In addition, individual susceptibility to cancer may result from host genetic factors. The NAT2 status is considered to be a risk factor for bladder cancer risk. However, some studies have failed to prove this relationship, which may be attributed to the statistical difference in sample size. Only a few studies exhibited a significant difference at the bilateral 5% statistical level. The fundamental basis of meta-analysis is to obtain a relative measurement of observation to support or deny certain assumptions, without directly combined with research findings. It is favorable to merge the collected data and analyze them with a variety of ways.

The allele frequency was analyzed to determine the influence factors of the disease, including race and geographical origin. However, the mix of clinical cases and controls in terms of race may occur in some of the studies. This mismatch between cases and controls indicates a source of the deviation. In addition, no polymorphic enzymes in carcinogenic metabolism can increase the risk of bladder cancer more than two times; a more realistic option would be to define the relevant risk as occurring <1.5 times. In addition, a majority of the published literature lacks power with respect to the NAT2 status (Sacks et al., 1987; Schnakenberg et al., 1998). Many published reports compare the NAT2 slow acetylation status among tumor patients and non-cancerous disease controls, as the authors theorized that the NAT2 slow acetylation state could be susceptible to non-cancerous disease. Numerous studies have investigated the possible association between the NAT2 status and the risk of bladder cancer. This meta-analysis, indicates a moderate correlation between the slow acetylation status and increased risk of bladder cancer, despite the heterogeneity. Although it is likely to be modest, a high frequency of NAT2 acetylation in a population could indicate a considerable influence on the incidence of bladder cancer. For example, 16% of the white race presented a 130-fold increased risk. In cases with a moderate (not high) expression of NAT2 in the bladder epithelium, rapid acetylation may protect bladder cancer metastasis to the liver and other organs through body detoxification (Sharpe S, 1998). Even when the polymorphic variation affects the genetic susceptibility of bladder cancer, there is a low possibility of the risk associating with any single locus. However, certain genotype combinations, such as the GSTM1 defects in the NAT2 slow acetylated combining state, may be greater risk factors than a single gene locus. Researchers are yet to reach a consensus regarding this so far (Taylor et al., 1998). A stricter level of significance is required for further subgroup analysis, and a larger sample size is required to clarify the impact of gene polymorphisms and the environment on bladder cancer.

Conflicts of interest

The authors declare no conflict of interest.

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