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Association between N-acetyltransferase 2 polymorphisms and pancreatic cancer risk: a meta-analysis

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ABSTRACT. N-acetyltransferase 2 (NAT2) is an essential phase II enzyme in the metabolism of aromatic and heterocyclic amines and of hydrazines. NAT2 activity can be divided into three phenotypes: rapid, intermediate, and slow. Studies identifying an association between NAT2 polymorphism and the risk of pancreatic cancer have shown conflicting results. In order to assess this relationship comprehensively, we performed a meta-analysis that involved 1607 patients with pancreatic cancer and 1682 controls from six studies, which were selected from a group of ten, identified by a search of PubMed and Embase databases up to July 2014. Relative risks (RRs) with 95% confidence intervals (CIs) were used to evaluate the relationships. In the overall analysis, no significant associations between *NAT2* rapid acetylation genotypes and pancreatic cancer risk (RR = 0.93, 95%CI = 0.73-1.19) were found; however, the results showed significant heterogeneity (I^2 = 55.0%).

The results from subgroup analysis suggested that the rapid genotypes might decrease the risk of pancreatic cancer (RR = 0.56, 95%CI = 0.38-0.84) in Turkey, although the association was not significant in the United States population (RR = 0.97, 95%CI = 0.71-1.34) or in the multi-center studies (RR = 1.10, 95%CI = 0.90-1.34). Analysis of the slow acetylation genotypes demonstrated the converse outcomes. In conclusion, the results of our study suggested that the *NAT2* slow acetylation genotypes might increase the susceptibility to pancreatic cancer in Europe but that these have no significant effects in the United States and multi-center populations.

Key words: NAT2; Polymorphism; Pancreatic cancer; Susceptibility; Meta-analysis

INTRODUCTION

Pancreatic cancer is a common malignant tumor and the fourth leading cause of cancer deaths in the United States (Siegel et al., 2013). Because of its insidious onset, the diagnosis of pancreatic cancer is usually delayed resulting in a poor prognosis for patients. Human pancreatic carcinogenesis is a complex and multigenetic process in which many factors such as age, gender, excess weight, diabetes, cigarette smoking, chronic pancreatitis, and family cancer history are involved (Michaud, 2004). Studies have shown that genetic polymorphism plays a significant role in the process of carcinogenesis. Genetic polymorphism has also been found to be associated with pancreatic cancer risk (Mazaki et al., 2011).

N-acetyltransferase 2 (NAT2) is an important phase II enzyme in the metabolism of aromatic and heterocyclic amines and of hydrazines (Liu et al., 2009). *NAT2* is a polymorphic gene located on chromosome 8p21.3-23.1 that encodes a 290-amino acid protein (Blum et al., 1990). The *NAT2* polymorphism has been classified into three phenotypes based on the allelic variant: fast (rapid), intermediate, and slow (Walraven et al., 2008). The alteration of the NAT2 acetylator status caused by the polymorphism might decrease its enzymatic activity and lead to the absence of efficient detoxification, further elevating cancer risk (Wikman et al., 2001; Cui et al., 2011). We hypothesized that such alteration of NAT2 enzyme activity might be involved in the susceptibility to pancreatic cancer.

Since Bartsch et al. (1998) first identified the relationship between NAT2 acetylator status and pancreatic cancer risk, several studies had been published that describe the association between *NAT2* genetic polymorphism and pancreatic cancer risk; however, none of these have provided conclusive results (Bartsch et al., 1998; Li et al., 2002, 2006; Jiao et al., 2007; Ayaz et al., 2008; Suzuki et al., 2008). To quantitatively estimate precisely the potential effects of the NAT2 acetylator phenotype on pancreatic cancer risk, we conducted a meta-analysis.

MATERIAL AND METHODS

Publication search

We searched published studies in the PubMed and Embase databases updated to July 2014. The search was limited to studies reported in English by using the following search

terms: “N-acetyltransferase” or “acetyltransferase” or “N-acetyltransferase 2” or “NAT2” and “pancreas” or ‘‘pancreatic’’ and “cancer” or “neoplasms” or “carcinoma” or “tumor” or “adenocarcinoma”. Furthermore, reference lists of the main reports and review articles were also reviewed to identify additional relevant publications.

Selection criteria

Two authors independently reviewed the retrieved titles and abstracts to determine the eligibility of the studies for inclusion in our meta-analysis. Published studies were included based on the following criteria: 1) written and published in English; 2) used case-control design; 3) concerned the association of NAT2 polymorphism with pancreatic cancer; and 4) presented sufficient data to calculate the relative risk (RR) estimates and their 95% confidence intervals (95%CIs). We excluded studies with the following criteria: 1) written and published in a language other than English; 2) no control group; 3) no usable information reported; 4) not human studies; or 5) reviews and duplicated studies.

Data extraction

Two investigators independently performed the data evaluation. The following data were extracted from each study: last name of the first author; publication year; study location; patients with rapid, intermediate, and slow acetylation status; and controls with rapid, intermediate, and slow acetylation status.

Data synthesis and statistical analysis

NAT2 polymorphisms were analyzed as dichotomous variables, as rapid versus intermediate and slow in the analysis of rapid acetylation genotypes, or as slow versus intermediate and rapid in the analysis of slow acetylation genotypes. The data of the patients and controls with rapid, intermediate, and slow acetylation were extracted and calculated using the initial data of the studies. These data were analyzed by the random-effect method, and were measured by RRs with 95%CIs. Statistical heterogeneity was estimated by means of the Cochran’s Q-test and the *I*-squared test. The *I*-squared test represented the percentage of variation for heterogeneity, which is categorized as low (0-40%), moderate (40-60%), high (60-90%), or very high (>90%). Subgroup analyses were carried out based on study location. To identify any potential publication bias, we used Begg and Egger tests; the results of these tests are not shown. All statistical analyses were performed with Review Manager 5.2 (The Nordic Cochrane Centre, Copenhagen, Denmark) and STATA 12.0 (StataCorp., College Station, TX, USA).

RESULTS

Systematic review

We identified 883 studies that fit our search strategy, but only 10 matched with the inclusion criteria and content (Figure 1). After reviewing the full texts, four studies were excluded following our search terms (Gross et al., 1999; Agundez, 2008; Brand et al., 2010; Jang

et al., 2012). Finally, we identified six studies for the meta-analysis (Bartsch et al., 1998; Li et al., 2002, 2006; Jiao et al., 2007; Ayaz et al., 2008; Suzuki et al., 2008).

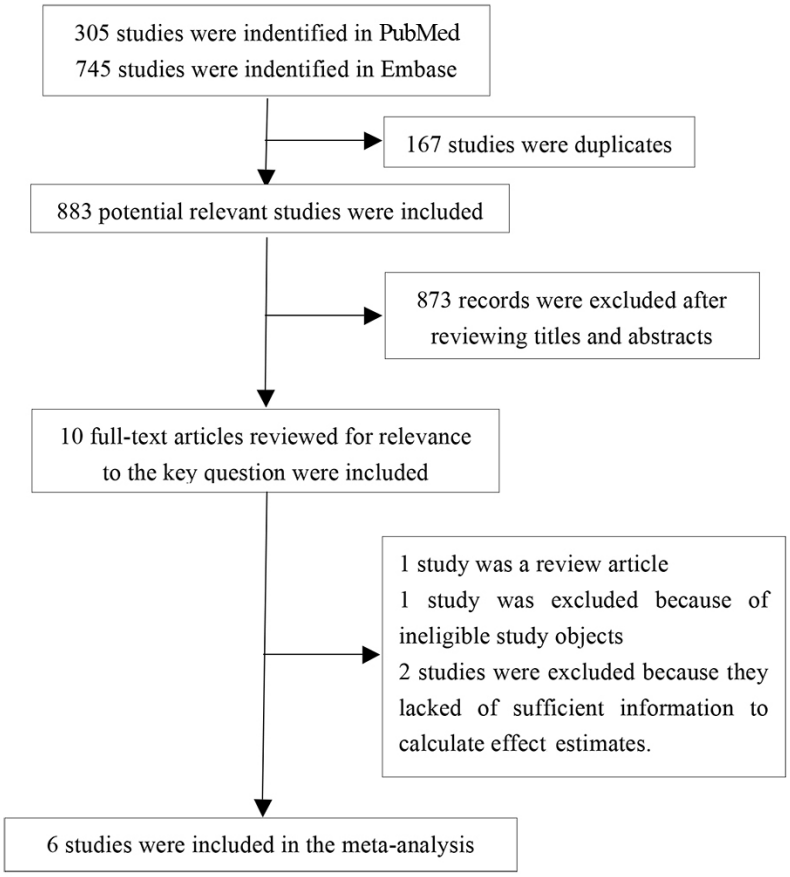

Figure 1. Flow diagram of the included/excluded studies.

Detailed characteristics of these studies are provided in Table 1. The studies included were published between 1998 and 2008, and all were case-control studies. These studies together included 1607 patients and 1682 controls. In the patient group, 162 carried rapid NAT2 polymorphisms, 540 were intermediate, and 905 were slow. In the control group, 236 were rapid, 529 were intermediate, and 917 were slow. The study locations included three studies performed in United States (Li et al., 2006; Jiao et al., 2007; Suzuki et al., 2008), one in Turkey (Ayaz et al., 2008), and two multi-center studies (Bartsch et al., 1998; Li et al., 2002). Regarding the division of NAT2 phenotypes, three studies utilized three phenotypes (rapid, intermediate, and slow) (Li et al., 2006; Jiao et al., 2007; Suzuki et al., 2008) and other three studies divided the phenotypes into two categories (rapid and slow) (Bartsch et al., 1998; Li et al., 2002; Ayaz et al., 2008).

Table 1. Characteristics of studies included in the meta-analysis.

Last name of first author	Publication year	Study location	No. of patients			No. of controls		
			Rapid	Intermediate	Slow	Rapid	Intermediate	Slow
Bartsch	1998	Multi-center	38	-	43	33	-	45
Li	2002	Multi-center	25	-	5	26	-	8
Li	2006	United States	17	97	181	27	105	185
Jiao	2007	United States	31	195	299	27	211	326
Ayaz	2008	Turkey	13	-	14	89	-	15
Suzuki	2008	United States	38	248	363	34	213	338

Study subject numbers are listed by the rate of acetylation associated with their different N-acetyltransferase 2 genotypes (Walraven et al., 2008).

Rapid acetylation genotypes

In the meta-analysis, six studies were included that examined the association between the NAT2 polymorphism associated with rapid acetylation genotypes and pancreatic cancer risk (Bartsch et al., 1998; Li et al., 2002, 2006; Jiao et al., 2007; Ayaz et al., 2008; Suzuki et al., 2008). The study-specific RRs for the NAT2 polymorphism associated with rapid acetylation genotypes ranged from 0.56 to 1.23. The overall RR of pancreatic cancer for the rapid phenotypes was 0.93 (95%CI = 0.73-1.19), with significant heterogeneity (I^2 = 55.0%) (Figure 2). In subgroup analysis, we found that the pooled RR for the United States studies was 0.97 (95%CI = 0.71-1.34) with low heterogeneity (I^2 = 15.2%). The RR for the multi-center studies was 1.10 (95%CI = 0.90-1.34) with low heterogeneity (I^2 = 0.0%). Only one study was based in Turkey, so we therefore could not calculate the pooled RR; the RR of this study was 0.56 (95%CI = 0.38-0.84). A Begg's funnel plot (P = 0.348) and an Egger's plot (P = 0.454) were created for assessment of possible publication bias. Both suggested that the publication bias had little influence on the results of this meta-analysis.

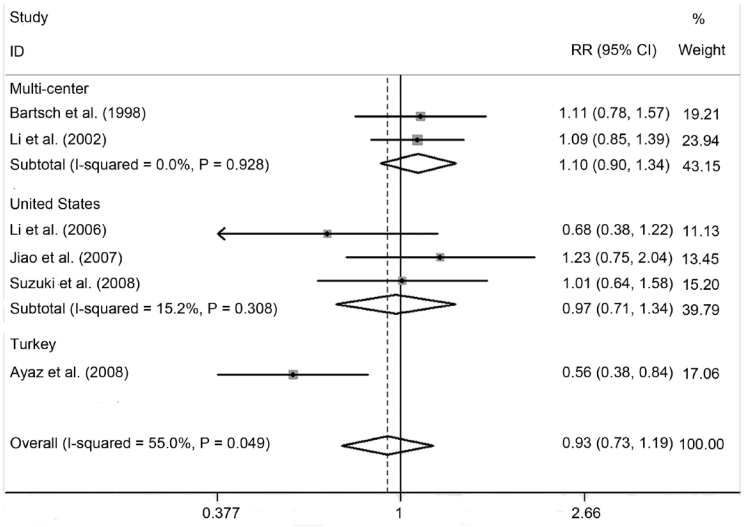

Figure 2. Forest plot of the meta-analysis using a random-effect model for the association of pancreatic cancer risk with the rapid acetylation N-acetyltransferase 2 genotypes. The dotted line reflects the pooled RR of included studies. The shaded boxes reflect the weighting of included studies. RR = relative risk.

Slow acetylation genotypes

The study-specific RRs for the NAT2 polymorphism associated with the slow acetylation genotypes ranged from 0.71 to 3.60. The overall RR of pancreatic cancer for the slow acetylation genotypes was 1.05 (95%CI = 0.90-1.22), with significant heterogeneity (I^2 = 74.7%) (Figure 3). In subgroup analysis, we found that the pooled RR for the United States studies was 0.99 (95%CI = 0.93-1.06), with low heterogeneity (I^2 = 0.0%). The RR for the multi-center studies was 0.90 (95%CI = 0.69-1.18), also with low heterogeneity (I^2 = 0.0%). As only one study was based in Turkey, we could not calculate the pooled RR; the RR of this study was 3.60 (95%CI = 1.99-6.50). A Begg's funnel plot (P = 0.573) and an Egger's plot (P = 0.370) were created for assessment of possible publication bias. Both suggested that the publication bias had little influence on the results of this genotype meta-analysis.

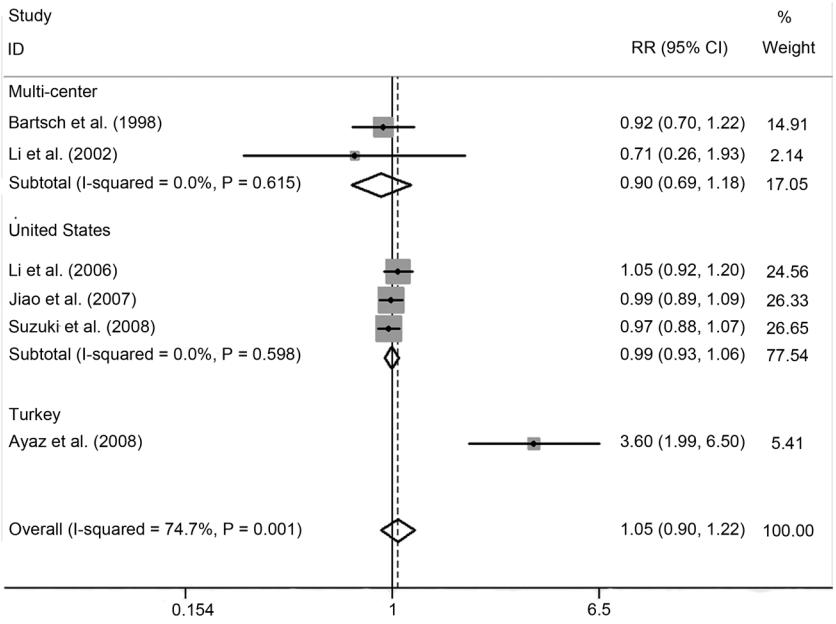

Figure 3. Forest plot of the meta-analysis using a random-effect model for the association of pancreatic cancer risk with the slow acetylation N-acetyltransferase 2 genotypes. The dotted line reflects the pooled RR of included studies. The shaded boxes reflect the weighting of included studies. RR = relative risk.

DISCUSSION

Tumor development is closely related to different carcinogenic factors within human organs. People have different susceptibilities to carcinogenic factors; this has been considered the result of genetic polymorphisms (Autrup, 2000; Vineis, 2003).

NAT2 is known to be involved in the metabolic processing of various potential carcinogens, and polymorphism in NAT2 was shown to have significant correlation with the risk of several types of cancer (Zhong et al., 2010; Cui et al., 2011; Liu et al., 2012; Zhuo et al., 2012). NAT2 catalyzes the detoxification or activation of aromatic and heterocyclic amine

carcinogens by N-acetylation detoxification or by O-acetylation activation (Liu et al., 2009). *NAT2* genetic variation might lead to an increased risk of different type of cancer (Walker et al., 2009). However, the questions of whether NAT2 polymorphism is significantly associated with the risk of pancreatic cancer and the identity of the mechanism underlying the potential association are still in need of further exploration. After a thorough search, we identified a series of articles that had been published on these issues, but no clear consensus had been reached.

To our knowledge, this is the first meta-analysis that has identified a correlation between NAT2 polymorphism and pancreatic cancer. We included six related articles with 1607 patients and 1682 controls. The results of our meta-analysis suggested that NAT2 polymorphism was not significantly associated with pancreatic cancer risk in the overall population, which was consistent with the conclusions reached by most of the studies included. However, according to our analysis, NAT2 polymorphism did not appear to be an individual factor that directly affected pancreatic cancer susceptibility, but was shown to increase the risk of pancreatic cancer when combined or interacting with other genes. Gene combinations such as *NAT2* with *CYP1A2* or *GSTT* have been previously reported to be closely related to cancer susceptibility (Lang et al., 1994; Welfare et al., 1999; Yoshida et al., 2007). Such research on gene combinations and interactions can contribute to a more accurate evaluation of the epidemiology of pancreatic cancer susceptibility and to the exploration of the molecular biologic mechanisms of NAT2.

Gene-gene interaction is not the only means of influencing the susceptibility to pancreatic cancer; other external components including social and environmental factors can also interact with genes and play important roles in tumor development. We therefore conducted a subgroup analysis to identify whether interaction between external factors and NAT2 polymorphism significantly increased the risk of pancreatic cancer. Due to the limitations of the studies included, only racial factors were analyzed. We found that the influence of NAT2 polymorphism in cancer susceptibility varied significantly among different races and ethnicities. The racial factor might contribute the most to the possible presence of heterogeneity among these studies. In this meta-analysis, we found that the slow acetylation genotypes increased the risk of pancreatic cancer in Turkey. Conversely, the acetylation genotypes had no association with the risk of pancreatic cancer risk in the United States. Furthermore, the rapid acetylation genotypes increased the risk of pancreatic cancer albeit not to a significant degree in the multi-center studies. In recent years, many countries have participated in multi-center studies; however, the inclusion of other ethnic populations might influence the ability to detect an association between NAT2 polymorphism and the risk of pancreatic cancer. Therefore, we concluded that it is highly possible that the association between NAT2 polymorphism and the risk of pancreatic cancer is dependent on the underlying ethnographic heterogeneity.

In this meta-analysis, potential publication bias was analyzed by the Begg's funnel plot and the Egger's plot. The results from these two methods both suggested that publication bias likely exerts little effects on these results.

Some limitations of this meta-analysis should be acknowledged. First, all of the published studies and papers that were included were written in English. Some related published or unpublished studies that meet the inclusion criteria were likely missed. Second, this meta-analysis was limited by the low number of studies included, and there were insufficient data on the factors that might influence the NAT2 polymorphism such as single nucleotide polymorphism sites, smoking, gender, and age to support the subgroup analysis. Third, in the subgroup analyses, only one study identified the relationship between NAT2 polymorphism

and pancreatic cancer risk in Europe, so the pooled RR could not be obtained. The inclusion of only a single study raised the possibility that the results might be biased, so we therefore needed more studies in Europe for further analysis. We found that NAT2 polymorphism had different effects on pancreatic cancer among Asians and Europeans (Zhong et al., 2010; Liu et al., 2012; Zhuo et al., 2012), but no studies on the relationship between NAT2 polymorphism and pancreatic cancer among Asians were found. Consequently, studies relating NAT2 and pancreatic cancer susceptibility should be carried out in Asia. Of the studies analyzed in this meta-analysis, three divided the patients into three phenotypes (rapid, intermediate, and slow) (Li et al., 2006; Jiao et al., 2007; Suzuki et al., 2008) whereas the other three studies only discriminated two phenotypes (rapid and slow) (Bartsch et al., 1998; Li et al., 2002; Ayaz et al., 2008). As we did not know what methods to use to represent the intermediate phenotype category in the two-phenotype studies, only the data of the rapid or slow phenotype individuals were taken from the first group of studies and compared to all subjects.

In conclusion, this meta-analysis suggested that NAT2 polymorphism was not significantly associated with pancreatic cancer risk. However, subgroup analysis demonstrated that the effects of NAT2 polymorphism on pancreatic cancer varied among different racial types. In Turkey, the slow acetylation genotypes might increase the risk of pancreatic cancer, although the NAT2 polymorphism had no significant effect in United States and multi-center studies. These findings require more research before any firm association can be established.

Conflicts of interest

The authors declare no conflict of interest.

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