N-Acetyltransferase 2 status and gastric cancer risk: a preliminary meta-analysis

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Abstract

In recent studies *N*- Acetyltransferase 2 (*NAT2*) genotype has been considered as a risk factor for developing gastric cancer, however with conflicting results among Asian and Caucasian populations. In order to clarify the influence of *NAT2* slow acetylation status on gastric cancer risk, a preliminary meta-analysis of published case-control studies was undertaken. The primary outcome measure was the odds ratio (OR) for the risk of gastric cancer associated with the *NAT2* slow genotype using a random effects model. Pooling the results from the 5 studies identified (771 cases, 1083 controls), an overall OR for gastric cancer risk associated with the *NAT2* slow genotype of 0.91 emerged (95% CI: 0.54-1.55). The result suggests that the *NAT2* slow genotype has probably no effect on the risk of gastric cancer. Additional epidemiological studies, based on sample sizes that are commensurate with the detection of small genotypic risks, are required to confirm these findings. Future studies may also help to clarify whether geographic differences exist.

Key words: gastric cancer, genetic epidemiology, N- Acetyltransferase 2, meta-analysis

Introduction

The global incidence of gastric carcinoma (GC) is declining, especially in Western countries due to the marked improvement in food processing and storage, a decline in the use of salted or smoked products and better general hygiene conditions.[1] Among the known risk factors for GC, dietary factors are of paramount importance in the early phases of its development.[1] In the last few years a great emphasis has been placed on the role of aromatic and heterocyclic amines in gastric carcinogenesis: they are abundant in smoked/preserved food and fried/barbecued meats especially those which are well-done. In order to become carcinogens, aromatic and heterocyclic amines need to be activated, this occurs via a cytochrome-P-450 mediated hydroxylation, followed by an *O*-acetylation catalysed by *N*-acetyltransferase (NAT1 or NAT2 enzymes).[2] *N*-acetyltransferase enzymes may be polimorphic, so susceptibility to cancer can be in part mediated by genetically determined differences in the effectiveness of the activation and detoxification of potential carcinogens.[3] In particular, homozygous/heterozygous combination of the functional wt *NAT2**4 allele characterizes the rapid acetylator genotype, while the absence of this allele defines the slow acetylator genotype.[4] Thus, individuals with higher or lower *N*-acetyltransferase activity may constitute genetic subpopulations who show higher or lower sensitivity to the effects of exposure to carcinogenic aromatic and heterocyclic amines. Several studies examining the risk of cancer in relation to *NAT2* status have been published, and recent meta-analyses conclude that slow acetylator individuals have a 40% increase in the likelihood of developing bladder cancer compared to those with rapid acetylators,[5] while colorectal cancer does not appear to be associated with *NAT2* status.[6] Since gastric mucosa expresses NAT2 activity,[6] some authors hypothesised that an inherited different ability in the acetylation of aromatic amines may have a role in gastric carcinogenesis. Since Oda et al. [7] first reported an association between *NAT2* status and GC in 1999, six studies have been published in the literature, with conflicting results.[8-13] In order to clarify the effect of *NAT2* status on the risk of developing GC, we carried out a quantitative meta-analysis of the research published up to the 31st of March, 2005.

Methods

The digital medical databases used for the search were MEDLINE and EMBASE. The key words used for the research were NAT or *N*-acetyltransferase or NAT2, gastric or stomach, cancer or carcinoma, without restriction on language. The experimental designs taken into consideration were: case-control studies with hospital controls (C-C hb) and population based case-control studies (C-C pb).

The time period considered included research articles published up to the 31st of March, 2005.

For the meta-analysis, the following inclusion criteria were considered:
- presence of a quantitative assessment of the relationship between *NAT2* and GC;
- an appropriate description of *NAT2* status in cases and controls;
- results expressed as relative risk (RR) or odds ratio (OR);
- studies with a 95% confidence interval (CI) for RR or OR, or with the possibility to calculate these measures if standard deviation (SD) values were present;
- english language used.

We compared the results of our literature search to the review articles found using the above mentioned databases. Each article was blinded according to the authors, institution and journals. Data extraction was conducted and entered into a database by two researchers independently, and for conflicting evaluations an agreement was reached following a discussion. For each blinded article, the following data were extracted: year, location, ethnicity; source of cases and controls; sex ratio and mean age (or range whenever possible) of cases and controls; the number of cases and control with *NAT2* slow genotype; ORs values with their 95% CI and potential confounders investigated in the study. The odds ratio of gastric cancer associated with *NAT2* slow versus rapid genotype (rapid acetylators include individuals with at least one *NAT2*4 functional allele, as reported elsewhere [4]) was estimated for each study. In order to detect potential sample size bias, ORs and 95% CI were plotted against standard errors for each study. In carrying out the meta-analysis, the random effect model was used, taking into account the possibility of heterogeneity between studies.[14] Statistical analysis was undertaken using the program RevMan,[15] release 4.2. We also computed the power 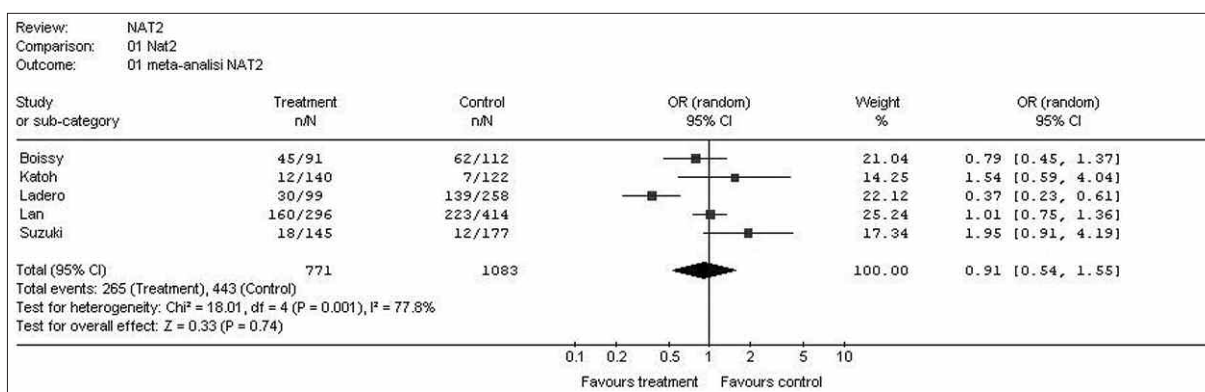of each selected study, in order to assess the probability of detecting an association between *NAT2* slow genotype and gastric cancer at the 0.05 level of significance, assuming a genotypic risk of 2 and 1.5, using the method described by Schlesselmann.[16]

Results

A total of seven articles were retrieved by our bibliographic search and two papers was excluded because one was written in a non-English language [13] and the other was later republished and included in our study.[7,8] Five case-control studies were therefore included for the meta-analysis, one was population based [11] and four were hospital based.[8-10,12] In all of the studies *NAT2* status was determined by analysis of the gene through polymerase chain reaction. In Table 1 the ORs are reported with 95% CI and the potential confounding factors that were analysed in each study. Age, sex, tumour feature and smoking habits were ascertained in all studies, alcohol consumption was verified in four studies,[9-12] others polimorphisms in three studies [8,9,11] and food consumption and *Helicobacter pylori* infection in only one.[11] Figure 1 shows the result of the pooled data, illustrating a plot of odds ratios (95% CI) for the risk of developing gastric cancer associated with *NAT2* slow genotype in the 5 case-control studies. The pooled analysis provided a non statistically significant result for the association between *NAT2* slow genotype and gastric cancer risk [OR of 0.91 (95% CI: 0.54-1.55; Q = 18.01)].

Unfortunately it was not possible to evaluate the potential interaction between *NAT2* status and smoking habits or diet, in relation to gastric cancer risk, because the raw data were not available. It is interesting to underline that Asian studies showed low power, and this could be due mainly to a low prevalence of *NAT2* slow genotype in the population. By examining the

Figure 1. Odds ratios (95% CI) for the risk of developing gastric cancer associated with *NAT2* slow status.
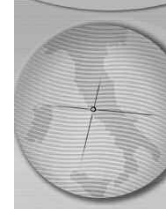

Study or sub-category	Treatment n/N	Control n/N	OR (random) 95% CI	Weight %	OR (random) 95% CI
Boissy	45/91	62/112		21.04	0.79 [0.45, 1.37]
Katoh	12/140	7/122		14.25	1.54 [0.59, 4.04]
Ladero	30/99	139/258		22.12	0.37 [0.23, 0.61]
Lan	160/296	223/414		25.24	1.01 [0.75, 1.36]
Suzuki	18/145	12/177		17.34	1.95 [0.91, 4.19]
Total (95% CI)	771	1083		100.00	0.91 [0.54, 1.55]

Total events: 265 (Treatment), 443 (Control)
Test for heterogeneity: Chi² = 18.01, df = 4 (P = 0.001), I² = 77.8%
Test for overall effect: Z = 0.33 (P = 0.74)

Table 1. Summary of studies of gastric cancer and *NAT2 status*.

Investigator	Place of study	Cases [a]	Rapid [#] n (%)	Slow [§] n (%)	Controls	Rapid [#] n (%)	Slow [§] n (%)	OR (95% CI) slow vs rapid	Confounders	Power RR= 1.5	Power RR= 2.0
Suzuki et al. 2004 [12]	Kanazawa and Komatsu, Japan	145 surgical cases from 2 hospitals; ages 62.4 (30-84); 66% male; Asian	127 (88)	18 (12)	177 cancer-free autopsy, age-matched; age at death 66.6 (20-93); 68% male; Asian	165 (93)	12 (7)	1.95 (0.85-4.49) [b]	age, sex, histotype, smoking habitus, alcohol, GSTM1, CYP2E1, p53 mutations	13%	41%
Lan et al. 2003 [11]	Warsaw, Poland	296 cases from 22 hospitals and 8 private endoscopic Units in the same city; histologically confirmed; ages NS; sex NS; Caucasian	136 (46)	160 (54)	414 population based (randomly selected among Warsaw resident); age and sex-matched; Caucasian	191 (46)	223 (54)	1.01 (0.74-1.37) [b]	Age, sex, education, pack-years of cigarette smoking, family history of stomach cancer, GST1 null genotype, years lived on a farm, fruit intake, anatomical site, histotype, diet before '90, childhood living conditions, history of selected medical conditions and medication (ulcer etc.) use, alcohol and other beverage ,lifetime occupational history, HP	72%	100%
Ladero et al. 2002 [10]	Madrid and Badajoz, Spain	99 incident and prevalent cases; histologically confirmed; ages 67.1 (13.4); 72% male; Caucasian	69 (70)	30 (30)	258 individuals: 217 young healthy volounteers; ages 36.3 (12.7); 54% male; 41 nonagenarians free of cancer and degenerative diseases; ages 92 (90-98) ; 24% male; Caucasian	19 (46)	139 (54)	0.37 (0.22-0.63) [b]	Age, sex, age at diagosis, anatomical site, histotype, smoking habits, alcohol	33%	76%
Boissy et al. 2000 [9]	North Staffordshire, UK	91 cases from 1 hospital; hstologically confirmed; ages 68; 72% male; Caucasian	46 (51)	45 (49)	112 hospital based cancer-free from the same hospital of cases; age 63; 52% male; Caucasian	50 (45)	62 (55)	0.79 (0.44-1.43) [b]	Age, sex, previous surgery, anatomical site, histotype, grading, differentiation, stadiation, IBD, smoking habits, history of cancer, marital status, alcool/drug intake, occupation	25%	61%
Katoh et al. 2000 [8]	Kitakyushu, Japan	140 cases from 3 hospitals; ages 61.6; 70% male; Asian	128 (91)	12 (9)	122 hospital-based healthy (check-up) in many clinics of the same city; ages 62.4; male 59%; Asian	115 (94)	7 (6)	1.54 (0.59 - 4.04) [b] 1.62 (0.61-4.58) [c]	Age, sex, smoking habits, grading	8%	23%

[a] Ages of cases and controls: mean (SD) or range given wherever possible
[b] Crude OR
[c] Adjusted OR for age, sex and smoking habits

[#] With at least one functional allele (NAT2*4)
[§] Without a functional allele (NAT2*4)

European and Asian studies separately, we obtained an OR of 0.68 (CI 95%: 0.37 – 1.25) and OR of 1.78 (CI 95%: 0.98 – 3.24), respectively.

Discussion

Most of the cancer susceptibility genes identified to date are rare and highly penetrant. While the individuals with rare alterations of these genes (e.g. tumor suppressor genes) have a dramatically higher risk of cancer, more common differences in the low penetrant susceptibility of genes (e.g. drug metabolism enzymes) could be responsible for a relatively small, but frequent increase in gastric cancer risk at the population level.[4,17] Although gastric cancer is one of the most common malignancies worldwide, the pathogenesis of this disease and the molecular genetic events that contribute to its development are poorly understood. Given that exposure to carcinogens is one of the most important risk factors for gastric cancer, the hypothesis that the modulation of carcinogen metabolism due to inherited polymorphisms in drug metabolism genes, could be a plausible way for explaining interindividual susceptibility.[17,18] Among the most recently studied metabolic gene polymorphism is the NAT2 gene, involved in acetylation of aromatic and heterocyclic amines. This conjugation reaction usually results in detoxification of these carcinogens (phase-II enzyme reaction), thus slow acetylator individuals could be more susceptible to those compounds. Since Oda et al. in 1999 [9] first drew attention to a possible relationship between NAT2 slow genotype and GC risk, further reports have been published examining this hypothesis,[8-13] however with conflicting results. This led us to undertake this preliminary meta-analysis, which aims to derive an estimate of the GC risk associated with NAT2 slow genotype. The main finding of this meta-analysis of five case-control studies is that the NAT2 slow genotype seems to be unrelated to gastric cancer risk (OR= 0.91; 95% CI: 0.54-1.55). The results are different when Asian and Caucasian populations are considered separately. If the slow genotype impacts on the risk of GC in the presence of aromatic and heterocyclic amines exposure by detoxificating those carcinogens, then the difference in the prevalence and intensity of such exposures might explain the results among Asian and Caucasian populations. In this context, we have to take into account that NAT2 activity often results in activation of carcinogens, thus conflicting results may depend on the prevalent enzymatic activity. Since NAT2 is presumed to confer susceptibility to GC via an interaction with carcinogens, it is interesting to remark that no data

on food consumption or exposure to other environmental carcinogens was collected from the cases or the controls in most of the studies.

The main limitations of this study have to be considered in interpreting the results. First, it was not possible to eliminate the principal form of bias in the study, that is the presence of potential confounding factors, as only a few studies reported total estimates that were adjusted using multivariate analysis. Secondly, only published studies were included in the pooled analysis. Therefore, publication bias may have occurred. Regarding this type of bias, a funnel plot indicated no evidence of publication bias (figure not shown). Usually positive results have a greater probability of being published with respect to negative ones,[19] which may have lead to an overestimation of the NAT2 null effect. Ideally, quality scoring should be used in order to determine which studies should be included in each meta-analysis.[20] This was not undertaken because the existing scales are yet to be validated, and it is also remains unclear how they could be readily applied to the published case-control studies on NAT2 status and gastric cancer.[21] Another bias to consider is selection bias, mainly due to a low power of the Asian studies. In future investigations, due to the low prevalence of the NAT2 slow genotype in these general populations, it will be necessary to sample cases and control 3-4 times higher than those previously selected, in order to achieve a power of at least 80%.[8,12]

Despite these limitations, from the results of this preliminary quantitative meta-analysis, that pooled the data from 1854 individuals (771 cases and 1083 controls), it appears that NAT2 slow genotype has probably no effect on the risk of gastric cancer. Future large epidemiological studies will be needed in order to gain a clearer picture of how this gene contributes to the development of gastric cancer, highlighting the interaction between risk factors (smoking habits, alcohol drinking, food and drug consumption), NAT2 status, and other genes (e.g. Phase I enzymes and other detoxifying enzyme systems) that may work cooperatively with NAT2 to protect the genome from chemical damage.

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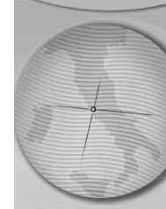
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