Available online at www.sciencedirect.com

ScienceDirect

Journal of the Chinese Medical Association 79 (2016) 105−110

www.jcma-online.com

Original Article

N-Acetyltransferase 2 (*NAT2*) genetic variation and the susceptibility to noncardiac gastric adenocarcinoma in Taiwan

Chih-Hao Chang [a,b,c], Yi-Shin Huang [a,b,*], Chin-Lin Perng [a,b], Han-Chieh Lin [a,b]

[a] *Division of Gastroenterology and Hepatology, Department of Medicine, Taipei Veterans General Hospital, Taipei, Taiwan, ROC*
[b] *National Yang-Ming University School of Medicine, Taipei, Taiwan, ROC*
[c] *Department of Gastroenterology, Zhongxiao Branch, Taipei City Hospital, Taipei, Taiwan, ROC*

Received June 15, 2015; accepted August 28, 2015

Abstract

Background: *N*-Acetyltransferase (NAT) is an important enzyme with the capacity to metabolize carcinogenic aromatic amines. However, it remains controversial whether the encoded functional *NAT2* genetic polymorphism is related to the risk of gastric adenocarcinoma (GA). The aim of this study was to evaluate the association between *NAT2* genetic variation and gastric adenocarcinoma (GA), with special reference to the gastric noncardiac adenocarcinoma (GNA).
Methods: Peripheral white blood cell DNA from 368 GA patients and 368 age- and sex-matched controls were genotyped for *NAT2* by a polymerase chain reaction method. The lifestyle habits of the participants were assessed using a semiquantitative food−frequency questionnaire. *NAT2* genotype, interaction with lifestyle habits, and the risk of GA and GNA were analyzed by logistic regression.
Results: GA patients were more likely to have a smoking habit, ate more salted foods, and consumed more well-done meat than the controls. There was no association between the *NAT2* genotypes and susceptibility to GA. However, if patients with gastric cardiac adenocarcinoma (GCA; $n = 42$) were excluded, the *NAT2* slow acetylators (without rapid acetylator allele) had a higher risk of GA than intermediate and rapid acetylators (odds ratio = 1.53; 95% confidence interval, 1.05−2.23, $p = 0.027$). In addition, there was a synergic effect of *NAT2* slow acetylator and well-done meat intake to the development of GNA (odds ratio = 3.83; 95% confidence interval, 1.68−8.76, $p = 0.001$).
Conclusion: *NAT2* slow acetylators have a higher risk of GNA than intermediate and rapid acetylators have in a Taiwanese population. The intake of well-done meat, an additive to the acetylator status, may contribute to the incidence of gastric carcinogenesis.

Keywords: arylamine *N*-acetyltransferase; gastric adenocarcinoma; gastric cancer; stomach neoplasms

1. Introduction

Aromatic amines (including heterocyclic amines and arylamines) formed from cigarette smoking and food cooked well-done are potent precarcinogens or carcinogens.[1] Aromatic amines are principally disposed of by *N*-acetyltransferase (NAT), in cooperation with a few phase 1or 2 enzymes.[2−5] The NAT enzymes are mainly encoded by the *NAT2* gene, which has many functional single nucleotide polymorphisms (SNP). The number of wild-type *NAT2*4* allele is divided in humans into rapid (2 alleles), intermediate (1 allele), and slow acetylators (none).[3,5] These different acetylator statuses may carry different individual susceptibilities to many cancers and diseases.[6−13] Among them, the association of gastric adenocarcinoma (GA) and *NAT2* genotypes has recently garnered much attention.[14−25]

GA is one of the most common cancers in many countries. The stomach is the primary gateway for nourishment, and thus

Conflicts of interest: The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

* Corresponding author. Dr. Yi-Shin Huang, Division of Gastroenterology and Hepatology, Department of Medicine, Taipei Veterans General Hospital, 201, Section 2, Shih-Pai Road, Taipei 112, Taiwan, ROC.
E-mail address: yshuang@vghtpe.gov.tw (Y.-S. Huang).

http://dx.doi.org/10.1016/j.jcma.2015.08.011

speculated to be exposed to many precarcinogens and carcinogens. It is also believed that the pathogenesis of GA is multifactorial and interactive with genetic and environmental factors.[26] Although heterogeneity exists in earlier studies, a meta-analysis has shown no association between *NAT2* polymorphism and GA susceptibility.[24] However, a more recent meta-analysis suggested that *NAT2* acetylator status has an effect on the risk of GA among East Asians.[25] In addition, lifestyle habit is a crucial environmental factor, which may interact with genetic factors to promote the development of GA.[17−22] The information of lifestyle habit and other confounding factors was controversial and complex in the *NAT2* gene−GA association studies.[17−22] Furthermore, gastric cardiac adenocarcinoma (GCA) was believed to have different characteristics and risk factors from gastric noncardiac adenocarcinoma (GNA).[27−29] However, all prior relevant literature did not further analyze the subgroups of GCA and GNA.[14−25] The aims of this study were to explore the relationship between *NAT2* genetic variation and GC in a Taiwanese population, with special reference to GNA, and also to investigate the gene-environment interactions with different lifestyle habits to the susceptibility of GA.

2. Methods

2.1. Study population

A total of 368 consecutive patients with pathology-confirmed gastric adenocarcinoma were prospectively enrolled in this study from 2001 to 2005. The other 368 sex- and age-matched (±3 years) patients without GC were recruited as controls. These controls were the in- or outpatients of our hospital, who had received pan-endoscopy examination and the results turned out be no GA.

GCA was defined as the tumor located only in the cardiac region of the stomach, or tumors located primarily in the cardiac region with slight involvement of the fundus.[27]

The participants were interviewed after written consent was obtained, and they completed an abbreviated food frequency questionnaire. Those patients who declined to give consent or who failed to answer the questionnaire were excluded. This study protocol was approved by the Institutional Review Board of Taipei Veterans General Hospital, Taipei, Taiwan.

2.2. Lifestyle evaluation

The lifestyle evaluation of this study was mainly focused on dietary habits. These habits were assessed using a semi-quantitative food frequency questionnaire, modified from a previously validated instrument.[10,30] This questionnaire included common food items with specified serving sizes that were described using natural portions of standard weight and volume measures of the servings commonly consumed in this study population. Cue cards were used to help identify serving sizes of individual food. Participants were asked how often, on average over the past year, they consumed that amount of each food. Participants chose one of seven frequency categories, which ranged from “Never” to “Six or more times daily”. The selected frequency categories for most food items were converted to a daily intake. As the average level of alcohol consumption in Taiwan is modest, habitual alcohol drinking was defined as consuming at least 30 g of alcohol, on average, daily for >10 years.

2.3. NAT2 genotyping

DNA was isolated from frozen white blood cells of the participants. The *NAT2* genotype was determined by the SNP-specific polymerase chain reaction (PCR), adopted from the studies by Hein and Doll.[31,32] This method can identify the seven most frequent SNPs: 191G > A (rs1801279), 282C > T (rs1041983), 341T > C (rs1801280), 481C > T (rs1799929), 590G > A (rs1799930), 803A > G (rs1208), and 857G > A (rs1799931). The wild-type allele detected was *NAT2*4*. Both *NAT2*4* and *NAT2*13* were regarded as rapid acetylator alleles, with other genotypes noted as slow acetylator alleles.[1,2] The presence of any two slow acetylator mutant alleles defines the slow acetylator genotype, whereas intermediate and rapid acetylators have one and zero slow acetylator alleles, respectively. Laboratory personnel were blinded to the case−control status.

2.4. Statistical analysis

Expected gene frequencies were calculated from respective single allele frequencies using the Hardy−Weinberg equation, wherein the observed and expected gene frequencies were compared using the chi-square goodness-of-fit test. Chi-square test was used for categorical data. The odds ratio (OR) with a 95% confidence interval (CI) of the possible risk factors for GA was calculated by logistic regression. The effect of modifying the relationship between the *NAT2* acetylator status and GA by lifestyle habit was assessed using a multivariate logistic regression analysis. This was done to compare the goodness of fit of the model containing an interaction term (*NAT2* acetylator status × lifestyle habit) with a reduced model containing indicator variables of the main effects of acetylator status and lifestyle habit.[10] Overall survival (OS) was estimated from survival curve based on the Kaplan−Meier method, and the log−rank test was used to compare the OS between different *NAT2* acetylator statuses. Statistical tests were based on a two-tailed probability. A p value < 0.05 was considered significant. All of the above-mentioned analyses were performed using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). In addition, G*Power 3.1.7 (Heinrich Heine University Düsseldorf, German) was used to estimate the required sample size. Under the assumption of effect size = 0.15, $\alpha = 0.05$, and power = 0.80, the total sample size was 349.

3. Results

Among the lifestyle risk factors, habitual alcohol drinking, vegetable, fruit consumption, and Lauren's histological type were not shown to be associated with GA (Table 1). However,