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Effects of Dietary Factors and the NAT2 Acetylator Status on Gastric Cancer in Koreans

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Abstract

Environmental dietary carcinogens and genetic polymorphisms in metabolic enzymes have been reported to be risk factors for gastric cancer. This study was undertaken to investigate the effects of the diet, the N-acetyltransferase (NAT) 2 acetylation status, and their interaction on gastric cancer risk. The study population consisted of 471 gastric cancer patients and 471 age- and sex-matched control subjects. NAT2 genotypes were identified using single-nucleotide primer extension reaction methods. Thirty-one alleles related to 12 polymorphism sites were assayed in this study. Significantly increased odds ratios were observed in former smokers (OR = 2.39, 95%CI = 1.57-3.62), heavy drinkers (OR = 1.28, 95%CI = 1.06-1.55), and individuals who eat well-done meat (OR = 1.24, 95%CI = 1.09-1.41). The odds ratios (95% CI) for high intake of kimchi, stews, and soybean paste were 3.27 (2.44-4.37), 1.96 (1.50-2.58), and 1.63 (1.24-2.14), respectively. The NAT2 genotype alone was not associated with gastric cancer risk. A significant gene-environment interaction was observed between environmental carcinogens and NAT2 genotypes. The odds ratios for kimchi, stews, and soybean paste were higher in slow/intermediate acetylators than in rapid acetylators. The odds ratios for slow/intermediate acetylators were 2.28 (95% CI: 1.29-4.04) for light smokers and 3.42 (95% CI: 2.06-5.68) for well-done meat intake. The NAT2 acetylator genotype may be an important modifier of the effects of environmental factors on gastric cancer risk.

Keywords

gastric cancer; N-acetyltransferase 2; genotype; dietary factor

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Background

Gastric cancer remains the second most common cancer in incidence and mortality worldwide, despite its overall decline.1 Asian countries with a particularly high incidence of this disease include Japan, Korea, and China. In Korea, gastric cancer is the most common cancer. In addition to *Helicobacter pylori* infection, many other environmental factors, including a high level of alcohol intake, use of tobacco products, consumption of dietary carcinogens, and nutritional status appear to be important components of gastric cancer risk. Lifestyle factors, especially dietary factors, are thought to be important in modifying the risk of gastric cancer. Although the findings are not always consistent, most studies on the relationship between the diet and gastric cancer suggest that foods rich in nitrate or nitrite, a high salt diet, and well-done meat increase the risk of gastric cancer,2–5 whereas frequent consumption of foods rich in antioxidants, including vitamins C and E, may decrease the risk of gastric cancer.6, 7 The dietary habits of Koreans can be characterized by a high intake of salty foods and smoked or barbecued meat. Experimental data have shown that prolonged cooking of meat at a high temperature favors the production of several potent carcinogens, including heterocyclic amines (HCAs),8, 9 which are also found in cigarette smoke.10 Well-done meat contains 10 times the concentration of HCAs in rare meat prepared using the same cooking methods.11 HCA carcinogens require host-mediated metabolic activation before initiating DNA mutations that progress to tumors in target organs.12 N-acetyltransferase (NAT) is a critical enzyme in the activation and detoxification of these carcinogens.13 The distribution and levels of NAT expression in humans are tissue specific: NAT1 is present in most tissues throughout the body, whereas NAT2 is expressed predominantly in the liver and gastrointestinal tracts.14 Correspondingly, the effect of exposure to heterocyclic amines on cancer risk appears to be organ specific.15 The NAT2 acetylator status may more effectually modify individual responses to various chemical carcinogens in gastrointestinal tract, and thus, may modify individual susceptibility of gastric cancer. NAT2 capacity varies among humans, and is often subdivided into rapid and slow, or rapid, intermediate, and slow acetylator genotypes.16, 17 Individuals with a slow acetylator status have reduced detoxification capacity compared with those with a rapid or intermediate status. Molecular epidemiologic studies have investigated the relationship between the NAT2 acetylator status and individual risks of cancer in various organs, including the urinary bladder,18 the colorectum,19 the breast,20 the prostate glands,21 the lungs,22 and gastric cancer.23 Except for smoking-related urinary bladder cancer most likely due to aromatic amine exposures,18 these studies have been unable to establish a consistent association between the acetylator status and human cancers. This hospital-based case-controlled study investigates the effects of the NAT2 acetylator status on gastric cancer risk and the interaction between the NAT2 acetylator status and exposure to environmental carcinogens.

Materials and Methods

Subjects

The cases studied were of Korean gastric cancer patients newly diagnosed with the disease from September 2000 to March 2005 at the Chungbuk National University Hospital and the Eulji University Hospital, which are located in the middle of the Korean peninsula. The control individuals were selected from patients diagnosed with diseases other than cancer in the same period and in the same two hospitals, and who matched the case subjects' gender and age (±3 years). To increase the comparability of the cases and controls, the controls were also selected from patients who were admitted to the Department of Orthopedic Surgery of the same hospitals from where the cases were chosen, due to bone fractures, osteoarthritis, or inflammatory bone disease. Individuals with a history of cancer, chronic

diseases due to their dietary intake pattern, or communication problems were not included in the control group.

Trained interviewers collected information on demographic factors and other known and potential risk factors for gastric cancer, using a standard questionnaire. As part of the interviews, the participants were asked to provide a detailed lifetime history of their tobacco and alcohol use and the usual degree of doneness of the meat they eat, as well as of their dietary information in the last 12 months preceding the interview. Dietary data were collected using a semi-quantitative food frequency table previously evaluated for validity and reliability. All the subjects were asked about the average frequencies of their intake and portion sizes of 89 common food items. These items were classified into 21 food groups with similar ingredients. The 21 food groups were as follows: cereals; potatoes; nuts; noodles; breads and cakes; vegetables; mushrooms; fruits; red meats; eggs; fishes and shellfishes; stews; chicken; kimchi; soybean foods; soybean pastes; milk and dairy products; butter, cheese, and margarine; jams, honey, candies, and chocolates; coffee and tea; seaweeds; and alliums.

Overall, 505 gastric cancer patients and 1,236 controls agreed to participate in the study. Among the 505 gastric cancer patients, 2 were excluded because gastric cancer occurred in two or more first and second degree relatives, and 32 were excluded who failed to match to controls. Consequently, the final case group included 471 patients. Of the 1,236 controls, 471 who were eligible for this study were matched to the cases as to age and gender. The means ± SD ages were 58.5 ± 10.6 years for the cases and 58.5 ±10.6 years for the controls. The distributions of the gender in the cases (315 men and 156 women) and the controls (315 men and 156 women) were identical. The Local Hospital Ethics Committee on Human Research approved this study, and all the participants gave their informed consent. Blood samples were collected from the cases and the controls and stored in a −80°C deep freezer.

Determination of NAT2 Genotypes

We extracted DNA from peripheral leukocytes using a commercial kit (DNA Extractor WB Kit, Wako, Japan). Seven hundred forty-eight fragments of *NAT2* that contained the polymorphic loci at nucleotide position numbers 190, 191, 282, 341, 364, 411, 481, 499, 590, 759, 803 and 857 of the *NAT2* coding region were amplified using the primers NAT2-fw, 5′-GAG GCT ATT TTT GAT CAC ATT-3′ and NAT2-rev, and 5′-ACA CAA GGG TTT ATT TTG TTC C-3′ in a 96-well plate thermal cycler (TaKaRa PCR Thermal Cycler Dice Gradient, Japan). A 25-ul reaction contained 50 ng of genomic DNA and 10× PCR buffer, 5 pmol of each primer, 200 uM of each dNTP, and 0.025 units of *Taq* polymerase. Thermal cycling was performed with an initial denaturation at 94°C for 5 minutes, followed by 35 cycles of 94°C for 25 seconds, 63°C for 45 seconds, 72°C for 50 seconds, and a final extension at 72°C for 7 minutes. To eliminate excess primers and dNTPs, the PCR product was purified with a purification kit (AccuPrep PCR Purification Kit, Bioneer, Korea).

The NAT2 genotype was determined using ddNTP primer extension assay. It was carried out using the ABI PRISM Snapshot ddNTP Primer Extension Kit (Applied Biosystems, USA), which contained fluorescently-labeled ddNTPs and DNA polymerase. Typing primers were designed to be annealed to the target DNA next to the 3′ end of the single nucleotide polymorphisms (SNPs). The primer sequences and the peak sizes of the Snapshot products are shown in Table 1. The SNP extension reaction was performed in 10-ul reaction with 1-ul purified PCR product, 4-ul Snapshot reaction mix, and 0.03 uM of each primer. Thermal cycling was performed with a rapid thermal ramp to 96°C for 10 seconds, 50°C for 5 seconds, and 60°C for 30 seconds, for 25 cycles. Then the reaction product was digested with shrimp alkaline phosphatase to inactivate the unincorporated fluorescently-labelled ddNTPs. The products of the single-nucleotide primer extension reaction were

electrophoresed with the ABI PRISM 310 Genetic Analyzer (Applied Biosystems) and analyzed using the GeneScan Analysis 3.1 software (Applied Biosystems) according to the manufacturers' instructions.

Thirty-five NAT2 alleles had been identified in the human population when this study was initiated. The reference allele is denoted as *NAT2*4*, and the 34 other variant alleles possess a combination of one to four SNPs at 15 sites within a 870-bp coding region. In this study, 31 alleles related to 12 SNPs were assayed. Based on recombinant enzyme expression data, five NAT2 alleles (*NAT2*4*, *NAT2*12A*, *NAT2*12B*, *NAT2*12C*, and *NAT2*13*) encode proteins with rapid *O*-acetylation capacities towards *N*-hydroxy amines, whereas the other NAT2 alleles encode proteins with reduced capacities.[24,25] Individuals who had two of the five rapid NAT2 alleles were classified as rapid acetylators; those with one of the said alleles, intermediate acetylators; and those who lacked them, slow acetylators.

Data Analysis

The amounts of calories, nutrients, vitamins, and minerals consumed with each food item were estimated by multiplying the amount of intake of the food item and its nutrient values. The total intake of calories, nutrients, vitamins, and minerals of each subject was calculated by summing up the calories, nutrients, vitamins, and minerals that corresponded to all the food items the subject consumed.[26] The amounts of intake of these factors were adjusted for caloric intake using the method of Willett et al.[27] We used the median values in controls as potential group cut-point for each of those variables. Alcohol drinking information on the questionnaire included average frequency of alcohol drinking (no drinking, 2-3 times per month, 1-2 times per week, 3-4 times per week, and daily) and average amounts. Alcohol drinking level was calculated by multiplying the average frequency of alcohol drinking and average drinking amount. Alcohol consumption was considered by groups of non-drinker, light drinker and heavy drinker, using ≥112 g ethanol/week as the cut off for heavy drinkers. Subjects who had smoked 200 cigarettes or more during their lifetime were classified as smokers; those with a history of smoking who had stopped at least 12 months before the interview were considered former smokers; those who had smoked fewer than 200 cigarettes were considered non-smokers. Pack-years were calculated according to the number of packs (20 cigarettes per pack) smoked per day multiplied by the number of smoking years. The meat doneness level was classified into three different categories: rare, medium-rare, and well-done.

Matched and unmatched analyses were conducted using conditional and unconditional logistic regression, respectively. Conditional logistic regression analyses with data, including all the matched case-control pairs, were performed to estimate the odds ratios and 95% CIs. The Hardy-Weinberg equilibrium for each polymorphism was tested separately for the cases and controls. The odds ratios and 95% CIs according to the NAT2 polymorphisms were estimated for each of the food groups using an unconditional logistic model that controlled the age, sex, and total energy intake, because matching of the cases and the controls in each NAT2 polymorphism group was not maintained. The homogeneities of the odds ratios according to the NAT2 polymorphisms were evaluated using the Breslow-Day test. *P*-values that were less than 0.05 were considered significant.

Results

The NAT2 genotype frequencies are shown in Table 2. The major genotypes were *NAT2*4/*4*, *NAT2*4/*6A*, and *NAT2*4/*7B*. Genotype distribution was consistent with Hardy-Weinberg equilibrium. There was no significant difference in the genotype distributions of the cases and the controls. The relative frequencies of the slow, intermediate, and rapid types among the cases (11.9%, 43.3%, 44. 8%) and the controls (12.3%, 45.7%, 42. 0%) were

similar. The OR for gastric cancer was 0.89 (95% CI 0.68-1.17) for the intermediate acetylators and 0.95 (95% CI 0.77-1.17) for the slow acetylators. Compared to the rapid acetylators, the OR for gastric cancer for the slow/intermediate acetylators was 0.94 (95% CI 0.83-1.08).

Table 3 shows the association among cigarette smoking, alcohol consumption, the degree of doneness of meat consumed, and gastric cancer risk, both overall and stratified according to the NAT2 acetylator status. There were significant differences between the cases and controls according to the smoking history, pack-years, alcohol intake, and well-done meat intake. Significantly increased odds ratios were observed in former smokers (OR = 2.39, 95%CI = 1.57-3.62), heavy drinkers (OR = 1.28, 95%CI = 1.06-1.55), and individuals who favored well-done meat (OR = 1.24, 95%CI = 1.09-1.41). Compared to the non-smokers as reference, the odds ratios for the light smokers and the heavy smokers were 1.70 (95% CI 1.11-2.60) and 1.39 (95% CI 1.13-1.70), respectively, both of which are statistically significant. Former cigarette smoking, heavy drinking and well-done red meat consumption showed higher risk estimates in slow/intermediate NAT2 acetylators than in rapid NAT2 acetylators. Among the light smokers, individuals with slow/intermediate NAT2 acetylator genotypes (OR = 2.28, 95%CI = 1.29-4.04) showed higher risks than those with rapid NAT2 acetylator genotypes (OR = 1.17, 95%CI = 0.62-2.22).

The analysis of the risks associated with dietary factors and stratified according to the NAT2 acetylator status is shown in Table 4. Compared to the controls, high consumptions of kimchi, stews, and soybean paste were associated with a higher risk of gastric cancer, whereas high consumptions of nuts, non-fermented alliums and soybean paste, and non-fermented seaweeds decreased the risk of gastric cancer. The odds ratios were 3.27 (95% CI 2.44-4.37), 1.96 (95% CI 1.50-2.58), and 1.63 (95% CI 1.24-2.14) for high intakes of kimchi, stews, and soybean paste, respectively. There was no significant difference, however, between the cases and the controls in the amount of their intake of fresh vegetables and fruits (data not shown). From the analysis of nutrient intakes, higher intake of vitamin B6 and iron were found in the controls than in the cases. There was a marginal inverse correlation between the total preformed vitamin B6 intake and gastric cancer risk. Stratified according to the NAT2 acetylator status, high consumptions of kimchi, stews, and soybean paste showed higher risks of gastric cancer in slow/intermediate acetylators than in rapid acetylators. The odds ratios for the slow/intermediate acetylators were 4.82 (95% CI: 3.23-7.19) for kimchi, 2.34 (95% CI: 1.64-3.34) for stews, and 1.82 (95% CI: 1.29-2.58) for soybean paste, respectively. The odds ratios for the rapid acetylators were 3.03 (95% CI: 2.00-4.62) for kimchi, 1.60 (95% CI: 1.07-2.38) for stews, and 1.42 (95% CI: 0.95-2.10) for soybean paste. High consumptions of nuts, non-fermented soybean foods, and seaweeds gave more protection against gastric cancer in the rapid NAT2 acetylators than in the slow/intermediate NAT2 acetylators. Among the nutrients, high intakes of vitamin B6 and iron gave protection against gastric cancer in the rapid NAT2 acetylators.

In the homogeneity test, the odds ratios of dietary carcinogens and alcohol consumption did not significantly differ in the slow/intermediate NAT2 acetylators and the rapid NAT2 acetylators, whereas the odds ratios of light smokers and well-done meat intake for gastric cancer between the slow/intermediate NAT2 acetylators and the rapid NAT2 acetylators were not homogenous. There were higher risks in individuals with slow/intermediate NAT2 acetylators for light smokers and well-done meat intake than with rapid NAT2 acetylators.

Discussion

Data from this study suggest that high consumption of foods rich in vitamin B6 and iron may reduce the risk of gastric cancer. Of all the vitamins, vitamin B6 is the most important

in the development and maintenance of a healthy immune system, and consequently protects against cancer and infection.29 Several epidemiological studies have shown the protective role of vitamin B6 against lung cancer, gastric cancer, and colorectal cancer.28¯30 It was found that individuals with a high dietary iron intake have a lower risk of gastric cancer. The results of this study show iron's protective role against gastric cancer, consistent with the results of previous prospective studies.31¯33 Broitman et al.34 suggested that initial chronic iron deficiency might lead to pre-malignant lesions such as chronic atrophic gastritis and achlorhydria, which favor bacterial colonization of the stomach and result in gastric carcinogenesis. In addition, prolonged iron deficiency caused by inadequate dietary iron intake or chronic blood loss through pre-malignant lesions may lead to changes in the gastrointestinal tract, which may impair the absorption of iron and other nutrients and, in turn, result in the development of diseases such as cancers. Iron has been reported to act as a cancer-promoting agent by producing "free radicals," and the production of free radicals is largely proportionate to the level of iron.35 Some studies have shown that people with high levels of iron have an increased risk for cancer.36،37

In this study, a decreased risk of gastric cancer was noted in individuals with a high consumption of nuts, non-fermented seaweeds, non-fermented soybeans, and alliums. Nuts are rich in unsaturated fatty acids, anti-oxidant vitamins, dietary fiber, and plant proteins, and contain many minerals, including iron, zinc, and magnesium. Many studies suggest that the consumption of nuts may reduce the risk of colon cancer and prostate cancer.38، 39

Epidemiological studies have shown that a higher intake of alliums is associated with reduced risk of several types of cancers. Some components of alliums block the metabolism of polycyclic hydrocarbons and nitrosamines, inhibit microbial activity, enhance immunocompetence, suppress cell division and proliferation, modulate phase I and II enzymes and DNA repair, and induce apoptosis.40 An animal study showed that alliums might suppress the growth of *Helicobacter pylori*.41 Eating fish has been reported to decrease the risk of gastric cancer in Japanese women42 and in Swedes.43 The effects of fish intake on the risk of gastric cancer varied according to the method of their preparation. Pan-fried fish decreased the risk of gastric cancer in Koreans, whereas stewed or boiled fish increased such risk.44

Soybeans are an abundant source of isoflavones45 and antioxidants,46 and have other anti-tumor effects, including the inhibition of angiogenesis,47 topoisomerase,48 and tyrosine kinase.49 An *in vitro* study has reported genistein's inhibition of the cell growth of stomach cancer cell lines.50 Thus, a high intake of soyfoods may reduce the risk of cancers. There are two main categories of traditional Korean soyfoods: non-fermented and fermented soyfoods. The main non-fermented soyfoods include soymilk, tofu (bean curd), and soybeans. The main fermented soyfoods include soybean paste and fermented soybeans, which generally have a high salt content.51 In a study of soybeans and cancer by Messina et al.,52 it was suggested that there is an inconsistent relationship between the intake of soyfoods and stomach cancer. The risk seemed to increase with the intake of fermented soyfoods (mainly *miso*) and to decrease with the intake of non-fermented soyfoods (mainly tofu). This study also showed that intake of non-fermented soybean products can reduce the risk of gastric cancer, whereas fermented soyfoods such as soybean paste can increase this risk.

Many epidemiological studies have reported a relationship between a high salt diet and gastric cancer.53¯55 Salt is thought to increase the risk of gastric cancer through damage to the gastric mucosa, which results in gastritis, increased DNA synthesis, and cell proliferation.56 Superficial gastritis can lead to chronic atrophic gastritis, which is a precursor lesion in the development of gastric cancer.2 Koreans have one of the highest rates

of 24-hour urine sodium excretion in the world,57 and the incidence of gastric cancer is very high among Koreans. It should be noted that a high salt diet is an important factor in gastric cancer development.

In this study, a high consumption of kimchi, soybean paste, and stews was associated with an increased risk of gastric cancer. Kimchi is Korean's traditional and favorite food. The major ingredient of kimchi is Chinese cabbage. Other common ingredients include cubed radishes and scallions. Kimchi is prepared with salted vegetables and dressed with the sauces containing garlic, ginger, scallions, radish, red pepper powder and jeot-gal. After preparation, this mixture is placed in clay containers then left to ferment slowly. Jeot-gal is made by various kinds of fishes or shell fishes, salted and then fermented in clay containers. Seel et al.58 found that the nitrate levels were significantly higher in kimchi (median: 1,550 mg/kg) than in jeot-gal (median: 140 mg/kg) ($P < 0.001$), and suggested that kimchi might play a role in gastric carcinogenesis among Koreans. Data from this study showed that high consumption of kimchi might increase risk of gastric cancer.

Most studies on the association between NAT2 genetic polymorphisms and cancer risk were performed among Caucasian populations in which the *NAT2*5B* allele is the most frequent slow acetylator allele, and two variant alleles, *NAT2*5B* and *NAT2*6A*, account for over 90% of the alleles associated with slow acetylators.59-61 The major slow acetylator alleles were *NAT2*6A* and *NAT2*7B* in Koreans. The frequency of *NAT2*6A* allele in Koreans (24% in our study) is similar with Caucasians (19-28%).59,60 Among Asians, the *NAT2*5B* allele is less prevalent and the *NAT2*7B* allele is more prevalent than among Caucasians.62 *NAT2*5B* is the most prevalent allele in Caucasians (40 %),63,64 but it occurs at a very low frequency in Japanese (0.5 %)65 and Koreans (1.9 % in our study). Our results show that *NAT2*7B* allele which is but rare in Caucasians (1-7%)66,67 is common (11.8%) in the Korean population.

Some publications have evaluated the relationship between NAT2 genotypes and gastric cancer risk.68-71 Three of them showed no association, and only one small case-control study reported a significantly increased risk from the combined intermediate and rapid NAT2 acetylation alleles versus the slow acetylation alleles. Gastric carcinogenesis is a complex process that results from interactions between genetic and environmental factors.72 Previous studies were not able to examine the interactions between the NAT2 acetylator status and dietary factors. This gene-environment interaction has strong biological plausibility, since slow NAT2 acetylators have a decreased capacity to detoxify arylamines via N-acetylation,13 and tobacco smoking and well-done meat intake are primary sources of exposure to arylamines in the general population. Consistent with this hypothesis, slow/intermediate acetylators have a decreased capacity to detoxify dietary carcinogen to reactive metabolites that initiate DNA adducts and tumors, compared with rapid acetylators. The study of Osawa et al.73 suggests that light smokers with slow/intermediate NAT2 activity had the highest risk of lung cancer among Japanese. Similarly, a trend towards increasing risk among light smokers with a slow acetylator status was seen in the study of Sillanpaa et al.74 In agreement with those studies, our study found an increased gastric cancer risk with smoking in slow/intermediate NAT2 acetylators. The highest risk was seen in individuals who had been smoking for less than 20 pack-years and who had a slow/intermediate NAT2 acetylator status. This is consistent with previous studies reporting that DNA adduct levels increase more significantly with lower exposure to nicotine-cotinine, particularly among slow acetylators.75

In our study, high consumption of kimchi, soybean paste, and stews was a risk factor for gastric cancer. To address the interaction between dietary factors and NAT2 genetic polymorphism on gastric cancer, the association of gastric cancer risk to the amounts of

intake of various foods, nutrients, vitamins and minerals according to the NAT2 status was statistically tested. It was found that high consumption of high-salt foods and well-done meat was associated with a higher risk of gastric cancer in slow/intermediate acetylators than in rapid acetylators. In contrast, high consumption of foods with protective roles against the disease was associated with a decreased gastric cancer risk only in rapid NAT2 acetylators. This study suggested that the slow/intermediate NAT2 acetylator genotype may have a decreased capacity to detoxify the carcinogens in the diet thus increasing gastric cancer risk in individuals with a high intake of foods that contain those carcinogens. From the homogeneity test, no significant difference was found between slow/intermediate and rapid NAT2 acetylators with respect to dietary factors and cigarette smoking. This should be further explored, however, with more epidemiological details in a larger study with the power to detect gene-environment interactions for gastric cancer.

The interaction between age and NAT2 genotype was demonstrated in various human cancers. Urinary bladder cancer risk was higher in older individuals than in younger individuals among slow acetylators in a study by Gu et al.76 In contrast, in a study of 124 Japanese non-small-cell lung cancer patients,77 a 3-fold relative risk for adenocarcinoma among younger individuals was observed among slow acetylators compared with rapid acetylators. This is consistent with a recent study in lung cancer of Slovak-Caucasians,78 that reported a tendency to adversely affect cancer risk in the individuals with the *NAT2*5B/*6* genotype in patients younger than 60 years (OR=3.14, 95% CI: 0.98-9.72). However, our study did not find the interaction between NAT2 genotype and age of gastric cancer onset.

This study has several strengths. It distinguished 12 NAT2 SNPs using the Snapshot primer extension method and DNA sequencing analysis. Most previous studies determined the NAT2 acetylator status based on four SNPs (481, 590, 803, and 857), which may lead to the misclassification of the genotypes. Moreover, most previous studies only investigated the role of the NAT2 genotype or the dietary factor in gastric cancer. This study examined the interaction between the NAT2 acetylator status and dietary factors to more clearly explain the role of the gene-environment interaction in gastric cancer. In summary, this study suggests that NAT2 acetylator status modifies the effects of dietary carcinogens and tobacco smoke on gastric cancer.

Conclusion

This study confirmed earlier findings of an increased risk of gastric cancer with exposure to dietary factors. Well-done meat and high consumption of kimchi, soybean paste, and stews were found to be risk factors, whereas high intakes of nuts, alliums, non-fermented seaweeds and soybeans, vitamin B6, and iron were found to protect against gastric cancer. An inherited deficiency in NAT2 metabolic capacity may be an important modifier of gastric cancer risk among Koreans with similar lifestyle factors.

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Table 1

Primer sequences used for multiplexed single nucleotide primer extension reaction and Snapshot products peak size.

SNP locus	Extension primer sequences	Direction	Product peak Size (bp)
NAT2-190	5′-AAAAAAAAAAAAAAAAAAAAAAAAATTTGATCACATTGTAAGAAGAAAC-3′	Forward	48
NAT2-191	5′-ACCTGGAGACACCACCCACCC-3′	Reverse	21
NAT2-282	5′-AAAAAAAAAAAAAAAAAAAAAAACAATGTTAGGAGGGTATTTTTA-3′	Forward	44
NAT2-341	5′-AAAAAAAAACACCTTCTCCTGCAGGTAACCA-3′	Forward	31
NAT2-364	5′-AAAAAAAAAAAAAAAAAAAAAAAAAAAAAATGACGGCAGGAATTACATTGTC-3′	Forward	52
NAT2-411	5′-AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAGATGTGGCAGCCTCTAGAATT-3′	Forward	56
NAT2-481	5′-AAAAAAAGAAGAGAGAGGAATCTGGTAC-3′	Forward	28
NAT2-499	5′-AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAATTCTTTGTTTGTAATATACTGCA-3′	Reverse	60
NAT2-590	5′-AAATACTTATTTACGCTTGAACCTC-3′	Forward	25
NAT2-759	5′-AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAATTATAAAGACAATACAGATCTGGT-3′	Forward	66
NAT2-803	5′-AAAAAAAAAAAAAAGAAGAGGTTGAAGAAGTGCTGA-3′	Forward	36
NAT2-857	5′-AAAAAAAAAAAAAAAAAAAAACTCGTGCCCAAACCTGGTGATG-3′	Forward	40

Table 2

Distribution of NAT2 acetylator genotypes among gastric cancer cases and controls

NAT2 genotype	Cases (n = 471)		Controls (n = 471)		OR (95% CI)
	No.	%[a]	No.	%[a]	
Rapid acetylators					
*NAT2*4 /*4*	211	44.8	193	41.0	
*NAT2*4 /*12A*	0	0	1	0.2	
*NAT2*4 /*13*	0	0	4	0.9	
Total rapid acetylators	211	44.8	198	42.0	1.0 (Reference)
Intermediate acetylators					
*NAT2*4 /*5B*	12	2.6	13	2.8	
*NAT2*4 /*6A*	120	25.5	126	26.8	
*NAT2*4 /*6C*	1	0.2	1	0.2	
*NAT2*4 /*7B*	70	14.9	74	15.7	
*NAT2*4 /*10*	0	0	1	0.2	
*NAT2*6A /*13*	1	0.2	0	0	
Total intermediate acetylators	204	43.3	215	45.7	0.89 (0.68-1.17)[b]
Slow acetylators					
*NAT2*5B /*6A*	4	0.9	3	0.6	
*NAT2*5B /*7B*	2	0.4	2	0.4	
*NAT2*6A /*6A*	16	3.4	22	4.7	
*NAT2*7B /*7B*	9	1.9	5	1.1	
*NAT2*6 A /*7B*	25	5.3	26	5.5	
Total slow acetylators	56	11.9	58	12.3	0.95 (0.77-1.17)[b]
NAT2 acetylators activity					
Rapid	211	44.8	198	42.0	1.0 (reference)
Slow / intermediate	260	55.2	273	58.0	0.94 (0.83-1.08)[c]

[a](participants with genotype/total cases or controls)*100.

[b]Unconditional logistic regression adjusted for gender and age.

[c]Conditional logistic regression stratified by age and gender.

Table 3

Association among cigarette smoking, alcohol consumption and doneness level of meat and gastric cancer risk, both overall and stratified by NAT2 acetylator status

	Total subjects		Rapid acetylators		Slow /intermediate acetylators	
	Case/Control	OR (95%CI)[a]	Case/Control	OR (95%CI)[b]	Case/Control	OR (95%CI)[b]
Cigarette Smoking (status at reference date and pack-years)[c]						
Non-smokers	173/208	1.0	83/92	1.0	90/116	1.0
Former smokers	167/115	2.39 (1.57-3.62)*	72/47	2.30 (1.21-4.37)*	95/68	2.51 (1.43-4.41)*
Current smokers	131/148	1.19 (0.97-1.46)	56/59	1.08 (0.82-1.50)	75/89	1.27 (0.95-1.69)
1-20 pack-years	98/93	1.70 (1.11-2.60)*	37/44	1.17 (0.62-2.22)	61/49	2.28 (1.29-4.04)e*
>20 pack-years	199/169	1.39 (1.13-1.70)*	91/61	1.44 (1.11-1.95)*	108/108	1.35 (1.02-1.80)*
Alcohol consumption[d]						
Non-drinkers	188/200	1.0	82/83	1.0	106/117	1.0
<112 g/week	108/148	0.83 (0.59-1.18)	51/66	0.78 (0.46-1.32)	57/82	0.87 (0.54-1.38)
≥112 g/week	173/123	1.28 (1.06-1.55)*	78/49	1.25 (0.93-1.68)	97/74	1.31 (1.01-1.68)*
Doneness level of meat						
Rare/Medium	346/349	1.0	164/139	1.0	182/210	1.0
Well-done	103/56	1.24 (1.09-1.41)*	33/30	0.94 (0.54-1.63)	70/26	3.42 (2.06-5.68) e*

[a] Conditional logistic regression stratified by age and gender.

[b] Unconditional logistic regression adjusted for gender and age.

[c] Pack-years were calculated as number of packs smoked per day multiplied by the number of smoking years; 20 cigarettes per pack.

[d] Ethanol uptake per week (g/week) means average ethanol uptake in gram times the average frequency of alcohol consumption per week in last 12 months.

[e] Odds ratio was significantly different between NAT2 slow acetylators and NAT2 rapid acetylators in a homogeneity test.

* p-value <0.05.

Table 4

Association between dietary factors and gastric cancer risk and stratified by NAT2 acetylator status.

	Total subjects		Rapid acetylators		Slow/intermediate acetylators	
	Case/Control	OR (95%CI)[a]	Case/Control	OR (95%CI)[b]	Case/Control	OR (95%CI)[b]
Nuts						
Low	335/243	1.0	154/101	1.0	181/142	1.0
High	136/228	0.44 (0.34-0.59)*	57/97	0.39 (0.25-0.59)*	79/131	0.47 (0.33-0.67)*
Stew						
Low	165/241	1.0	78/94	1.0	87/147	1.0
High	306/230	1.96 (1.50-2.58)*	133/104	1.60 (1.07-2.38)*	173/126	2.34 (1.64-3.34)*
Kimchi						
Low	106/241	1.0	55/101	1.0	51/140	1.0
High	365/230	3.27 (2.44-4.37)*	156/97	3.03 (2.00-4.62)*	209/133	4.82 (3.23-7.19)*
Soybean pastes						
Low	185/241	1.0	86/97	1.0	99/144	1.0
High	286/230	1.63 (1.24-2.14)*	125/101	1.42 (0.95-2.10)	161/129	1.82 (1.29-2.58)*
Nonfermented soybean foods						
Low	296/242	1.0	146/107	1.0	150/135	1.0
High	175/229	0.57 (0.43-0.75)*	65/91	0.45 (0.29-0.69)*	110/138	0.63 (0.44-0.91)*
Nonfermented alliums						
Low	287/245	1.0	131/105	1.0	156/140	1.0
High	184/226	0.71 (0.55-0.92)*	80/93	0.70 (0.47-1.04)	104/133	0.73 (0.51-1.03)
Nonfermented seaweeds						
Low	272/243	1.0	128/101	1.0	144/142	1.0
High	199/228	0.78 (0.60-1.03)*	83/97	0.67 (0.45-1.00)*	116/131	0.86 (0.61-1.22)
Sodium						
Low	158/236	1.0	76/100	1.0	82/136	1.0
High	313/235	2.14 (1.61-2.84)*	135/98	1.90 (1.27-2.85)*	178/137	2.30 (1.61-3.30)*
Vitamin B6						
Low	275/243	1.0	132/104	1.0	143/131	1.0

	Total subjects		Rapid acetylators		Slow/intermediate acetylators	
	Case/Control	OR (95%CI)[a]	Case/Control	OR (95%CI)[b]	Case/Control	OR (95%CI)[b]
High	196/236	0.71 (0.54-0.93)*	79/94	0.67 (0.45-1.00)*	117/142	0.75 (0.53-1.05)
Iron						
Low	263/235	1.0	125/97	1.0	138/138	1.0
High	208/236	0.77 (0.59-1.02)	86/101	0.67 (0.45-1.00)*	122/135	0.91 (0.64-1.28)

[a] Conditional logistic regression stratified by age and gender adjusted for total energy intake.

[b] Unconditional logistic regression adjusted for age, sex, total energy intake.

* p-value <0.05.