Published in final edited form as:

Cancer Lett. 2006 July 18; 238(2): 210–223. doi:10.1016/j.canlet.2005.07.004.

P53 Codon 72 polymorphisms: A case-control study of gastric cancer and potential interactions

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Abstract

P53 codon 72 polymorphisms have been reported to be associated with cancers of the lung, esophagus and cervix. However, there have been no reports on the interaction of select risk factors and p53 codon 72 polymorphisms in gastric cancer susceptibility. 155 gastric cancer cases and 134 cancer-free controls were enrolled at the Memorial Sloan Kettering Cancer Center (MSKCC) from November 1992 to November 1994. The crude odds ratio (OR1) associated with the (Pro/Pro) polymorphism and the risk of gastric cancer was 1.27 (0.70–2.33). Adjusting for age, sex, race and education (OR2) and further adjusting for BMI, calories, sodium, smoking, vitamin C, fiber, alcohol, fat, and H. pylori status (OR3) did not yield significant results. Significant joint effects were associated with high fat consumption (OR1 = 2.61 (95% CI:1.13–6.06); OR2 = 2.85 (95% CI:1.14–7.15) for total cancers and for proximal tumors (OR1 = 2.56 (95% CI:1.00–6.54)). The low vitamin C intake/high-risk polymorphism group (Pro/Pro) had an OR1 of 4.82 (95% CI: 1.72–13.45) and the OR2 was 6.19 (95% CI: 2.08–18.40) for distal tumors. The point estimates were

increased for interaction odds ratios but not statistically significant (OR1 = 4.25 (95% CI: 0.66–27.50); OR2 = 4.73 (95% CI: 0.67–33.43); OR3 = 5.55 (95% CI: 0.66–46.47)). Further studies specifically looking at proximal and distal tumors are required to confirm any potential interaction between the p53 codon 72 polymorphisms an environmental risk, in particular low dietary vitamin C and high fat consumption.

Keywords

Gastric cancer; p53 codon 72 polymorphism; gene-environment interaction

1. Introduction

Gastric cancer is an enormous international public health problem. It is the second most common fatal malignancy (after lung cancer), with 558,458 incident cases (10.5% of total cancers) and 405,215 deaths (11.5% of cancer deaths) annually worldwide [1]. Despite the overall decline in gastric cancer rates in most of the Western World, gastric cancer remains a serious fatal disease throughout much of the rest of the world. It still is the most frequent cancer in men (ahead of lung cancer) in tropical South America and high rates are also present in the former Soviet Socialist Republics and parts of Eastern Europe. Thirty-eight percent of worldwide cases occur in China, where it remains the most common cancer in both sexes as it is elsewhere in Eastern Asia [1]. Conversely, the incidence rates of adenocarcinomas of the proximal stomach (gastric cardia) and distal esophagus have been increasing, particularly in the Western World [2,3].

Gastric carcinogenesis is considered to be a complex, multi-step and multi-factorial process, where the response to various risk factors (particularly environmental) is modulated, in part, by inhibitory and stimulatory factors that are products of proto-oncogenes and tumor suppressor genes [4]. Chronic infection with *Helicobacter pylori* (*H. pylori*) has been considered a major environmental risk factor, and other reported risk factors (though the evidence is not quite as strong) include high sodium intake, smoked foods, high fat intake, cigarette smoking, obesity, increased dietary nitrite intake, and high alcohol intake [5–8]. Conversely, possible protective factors reported include increased intake of fresh fruits and vegetables, whole cereal grains, vitamins such as vitamin C and beta carotene, aspirin and/or non-steroidal antiinflammatory medications, minerals such as selenium, and green tea consumption [9–12].

With the advances in molecular biology, much progress has been made on the molecular pathogenesis of cancer. An important discovery was the p53 gene and the elucidation of its role as ‘the guardian of the genome’ by controlling for apoptosis and cell cycle arrest [13]. It has been associated with 60% of all cancers, and in gastric cancer, mutations and/or overexpression of the mutant p53 protein has been found in up to 76% of tumor samples [14]. Abnormalities of p53 have also been found in dysplastic tissue and intestinal metaplasia (a suspected precursor of gastric cancer). Further in vitro evidence has shown that mutated p53 genes, when transfected into gastric cell lines, did not suppress colony formation, whereas transfected wild type p53 will suppress colony formation [15]. Also,

there was less incorporation of thymidine into DNA with wild type p53 transfected cells, suggesting less cellular division. Recently, several common polymorphisms of the p53 gene have been described. One of these polymorphisms is the codon 72 polymorphism on exon 4. Interestingly, this area around exon 4 of the p53 gene encodes amino acids that lie close to the central hydrophobic area, which determines protein conformation and thus DNA-specific binding [16]. The polymorphism consists of a single base pair change of either arginine (CGC) or proline (CCC) which creates three distinct genotypes: homozygous for arginine (Arg/Arg), homozygous for proline (Pro/ Pro) and a heterozygote (Pro/Arg) [16]. The transcribed proteins for the polymorphisms have been shown to be different structurally by electrophoretic mobility assays and functionally in vitro, with the p53 (Pro/Pro) variant protein being a stronger inducer of transcription and showing slower kinetics in inducing apoptosis than the p53 (Arg) genotype; and with the p53 (Arg) type also suppressing transformation of primary cells to a higher degree than the p53 (Pro) type [17].

Human studies have shown that these polymorphisms are associated with an increased risk of developing certain tumors, and have an effect on outcomes. Individuals with the p53 Pro/Pro genotype have been shown to be more likely to develop lung cancer (especially in smokers), and to have slightly worse outcomes [18–21]. The Pro allele has also been found in increased frequency in breast cancer patients [22,23]. Conversely, recent studies with HPV-induced cervical cancer show that more cases have the homozygous Arg allele [24,25]. Both clinical and in vitro data suggest that p53 codon 72 variants may serve as risk factors for many different types of cancers, and may play a role in the modulations of certain environmental risk factors.

Few studies have examined the role of p53 codon 72 polymorphisms and gastric cancer. In a recent study from Japan, 117 cases of gastric cancer with *H. pylori*-induced chronic gastritis were compared with 116 cancer-free controls with *H. pylori*-induced chronic gastritis. It was found that the Pro/Pro allele was associated with an increased risk of developing diffuse type gastric cancer compared to the Arg/Arg (OR 2.98 CI: 1.07–8.32) [26]. In another study by Zhang et al., 120 cases were compared with 277 cancer-free controls. Using logistic regression analysis, and after adjustment for patient age and gender, their results showed that a homozygous patient (Arg/ Arg) has a risk for cardia cancer 3.1 (95% CI: 1.4–7.3) times greater than for Pro/Pro homozygous and Arg/ Pro heterozygous patients [27].

The primary aim of this study is to examine the effect of polymorphisms of the p53 codon 72 variant and the risk of acquiring gastric cancer. Secondary aims include the assessment of any interactions between the p53 codon 72 polymorphisms and select risk factors, and determining risk and interactions based on the location of cancer (distal vs. proximal tumors). The significance of this work is that this could stratify patients at risk for gastric cancer. Individuals with particular genotypes could be screened for prophylactic treatment of preventable risk factors such as *H. pylori* if it is found that there is a synergistic interaction between the polymorphism and known risk factors. This study will further increase the understanding of the etiology of gastric cancer. Not much is known about the distribution of p53 codon 72 polymorphisms in the U.S. population. Our study has the largest number of cases to examine the effects of codon 72 p53 polymorphisms. Thus far,

there have been no studies that have focused on codon 72 p53 polymorphisms and their interaction with environmental risk factors on the risk of gastric cancer.

To explore this question, we analyzed data from a hospital-based case-control study at the Memorial Sloan-Kettering Cancer Center conducted from November 1992 to November 1994. The first aim of this study was to investigate the risk of developing gastric cancer from the p53 codon 72 Pro/Pro polymorphism. We then investigated for any interactions between the Pro/Pro polymorphism and other environmental factors such as cigarette smoking, alcohol consumption, body mass index, and dietary intake of calories, fat, sodium, fiber and vitamin C. This was done for all the cases combined and by cancer site, which was classified as either distal esophagus/proximal stomach (cardia) cancer or distal cancer.

2. Materials and methods

2.1. Study subjects

A modified National Cancer Institute (NCI) self-administered Health Habits and History Questionnaire (HHHQ) was sent to 596 consecutive patients scheduled for an upper gastrointestinal endoscopic examination at the Memorial Sloan-Kettering Cancer Center from November 1, 1992 to November 1, 1994. Among the 596 patients, 483 (81%) returned their questionnaires and underwent endoscopic examination. Three hundred thirty-three patients were eligible and 295 individual blood samples were collected. Patients who participated in the study and had endoscopic examinations were later classified as cases or controls based on pathologic diagnosis. The sites of disease were classified using the International Classification of Diseases for Oncology [ICDO] and ICD-9-CM. For the purposes of this study, patients were assigned to either the normal control group or one of several different case groups: adenocarcinomas of the gastric cardia (ICD9 site code 151.0 and ICDO histology code 8140–8573), adenocarcinomas of the gastro-esophageal junction (ICD9 site code 151.0 and ICDO histology code 8140–8573), adenocarcinoma of the distal stomach (ICD9 code 151.1–151.9 and ICDO histology code 8140–8573), and chronic atrophic gastritis (ICD-9 code 535.1). All pathologic diagnoses were made after data was collected.

2.2. Data collection and nutrient estimation

A modified version of the NCI self-administrated Health Habits and History Questionnaire (HHHQ) was used to gather information about subjects' demographic variables (gender, race, year and place of birth, and education), personal habits (cigarette smoking, years of smoking, age at which the subjects began smoking, alcohol consumption, types and frequency of alcohol consumption), and health factors (occupational and environmental exposures, family history of cancer, and medical and dietary history). Information about specific food items, frequency of consumption, usual portion size, and restaurant use was also contained in this questionnaire. Nutrient intake and nutritional analysis were carried out using the HHHQ-Dietsys software and NCI algorithm (version 3.0; NCI, Bethesda, MD). More details on the nutrient estimates are described in our previous publication [27].

In order to measure serum antibodies against *H. pylori* and for genotyping, patients who participated in this study were also asked to donate 10-ml blood samples. At the time of analysis, all blood samples were stored at -70°C at the Molecular Epidemiology Laboratory of the University of California-Los Angeles Jonsson Comprehensive Cancer Center. All biopsies were performed utilizing an Olympus video endoscope (EVIS100) with at least two sets of endoscopic biopsies taken for histology from endoscopically normal and abnormal appearing gastric mucosa. A reference pathologist reviewed slides of 58 patients to assess the reliability of using the pathology reports for diagnosis. The agreement rate between the pathology reports and the results from the reference pathologist was greater than 90%; thus it was felt that the results from the pathology reports could be utilized without further confirmation of results by an additional reading. The diagnosis of *H. pylori* infection was based on the measurement of serum antibodies using a standard ELISA test. Among the original 596 patients who were sent questionnaires, 23 (3.4%) were ineligible because they were too ill or did not understand English, 6 (1%) died, 45 (7.5%) refused and 47 (7%) did not respond. The remaining 483 (81%) patients returned their questionnaires and received endoscopic examinations. Eighty-seven incident proximal gastric/distal esophageal cancers and 68 incident distal gastric cancers were diagnosed. One hundred thirty-four patients with negative endoscopies and who were cancer-free were selected as controls.

2.3. Genotyping

Genomic DNA was isolated from previously frozen whole blood by proteinase K digestion and phenol-chloroform extraction. Subsequent genotyping by a polymerase chain reaction (PCR)-restriction fragment length polymorphism assay was performed at the Molecular Epidemiology Laboratory in the UCLA School of Public Health. Two alleles encode either a CGC arginine (Arg) residue or a CCC proline (Pro) residue. The restriction enzyme BstU1 (New England Biolabs, Beverly, MA) recognizes the sequence 5'CG/GC3' or 3'GC/'. Three genotypes are possible: Arg/Arg, Arg/Pro and Pro/Pro. The Arg/Pro genotype results in the presence of two bands of 169 base pairs and 127 base pairs. The Arg/Arg genotype results in the presence of three bands of 169 base pairs, 129 base pairs and 296 base pairs, and the Pro/Pro genotype results in a single uncut band of 296 base pairs. The exact methods of PCR amplification have been described elsewhere [16]. The PCR results were evaluated without knowledge of the subjects' case/control status and all PCR results were repeated to confirm the genotypes.

2.4. Statistical analysis

The association between the p53 Pro/Pro polymorphism and the risk of gastric cancer was assessed by deriving odds ratios (ORs) and corresponding 95% confidence intervals (95% CIs) using a logistic regression model. First we assessed the association between the Pro/Pro polymorphism and the risk of developing gastric cancer regardless of tumor location or subtype (either distal or proximal). For each analysis, we calculated the crude ORs and adjusted ORs, controlling for potential confounding variables. OR₁ represents the crude unadjusted odds ratio; OR₂ is the odds ratio adjusted for age, sex, race, and level of education; and OR₃ is the odds ratio adjusted for variables in OR₂ plus alcohol consumption, vitamin C, calories, sodium, fat and fiber intake, and pack-years of smoking.

Before proceeding to interaction assessment, we first eliminated subjects with daily dietary caloric intake higher than 4,500 kcal or lower than 500 kcal from this analysis to reduce the chance of coding errors. In the next step for evaluating interactions, we categorized our main effect variables into two categories: exposed or unexposed; or low dose of exposure or high dose of exposure. Our criteria for categorizing each continuous variable, including BMI, calories, fat, sodium, fiber, and vitamin C, were based on the distributions of the cancer-free controls. The exposures were considered high if the amount of exposure exceeded 50% of the controls, while the exposures were considered low if they fell below 50% of the controls. For the analysis with smoking as the primary variable of interest, it was defined by the number of pack-years of smoking (0–20, 21–40, 41–60 and greater than 60 pack-years); otherwise it was used as a continuous variable. For alcohol consumption, beer, wine, and liquor were combined to yield the quantity of drinks per day.

To obtain the odds ratios of both main effects and joint effects for the p53 codon 72 polymorphism and the selected exposure, two categories were created, each of which contained one possible combination of p53 codon 72 status and exposure status, such as p53 codon 72 negative and exposure positive (or high). Being negative for p53 codon 72 means that the genotype was either heterozygous (arginine/proline) or homozygous for the arginine genotype. The high-risk or positive exposure was the homozygous proline genotype. These variables were fitted into a logistic regression model to obtain the OR estimates of each main effect and the joint effect. Similar to the preceding analytical strategies, effect estimates were also presented as crude (OR_1) and adjusted (OR_2) odds ratios controlling for age, sex, race, and education, as well as OR_3 , which is OR_2 with an adjustment for further potential confounders as listed in Table 2. Departures from additivity or multiplicativity were considered to indicate interaction effects.

A more than additive interaction was indicated when

$$OR_{11} > OR_{10} + OR_{01} - 1$$

where the subscripts indicate presence (₁) or absence (₀) of the categories with respect to the baseline ₀₀ state. A more than multiplicative interaction was indicated when:

$$OR_{11} > OR_{10}^* OR_{01}.$$

The corresponding 95% CIs for the departures from additivity were calculated based on the equation derived by Rothman; and the 95% CIs for the departures from multiplicativity were assessed by fitting the two main factors and an interaction term into the logistic regression model. Potential empirical confounders were controlled in the logistic regression model and treated as continuous variables when appropriate. All the statistical analyses were performed with Statistical Analysis System software (v.8.2, SAS Institute, Cary, NC).

3. Results

3.1. Descriptive statistics

292 blood samples were analyzed for this study, with three patient samples omitted because of additional cancer diagnoses (melanoma, uterine and ovarian tumors). A total of 155 cases of gastric cancer and 134 cancer-free controls were analyzed for this study. 87 patients (56.1%) had the diagnosis of proximal stomach/distal esophageal cancer while 68 patients (43.9%) had distal stomach cancer. Cases tended to be older, male, and self-reported to be Caucasian (see Table 1) as compared to the controls. The controls selected for this study were cancer-free and without gastritis as per ICD9 coding. The distributions of each p53 codon 72 polymorphism can also be seen in Table 1 between cases and controls. The distribution of the alleles did not violate the Hardy-Weinberg equation (results not shown) as the p-value was greater than 0.05, suggesting that despite being a referral-based population of patients, the alleles were still in Hardy-Weinberg equilibrium.

Table 2 shows the three different logistic regression odds ratios evaluating the high-risk Proline/Proline genotype with potential risk factors, divided by the type of cancer (i.e., proximal vs. distal vs. total). Univariate analysis of the Proline/Proline genotype did not show any significant effects as all the results crossed unity (Table 2). Controlling for other risk factors also did not uncover any significant associations.

3.2. Odds ratios for risk factors

There were several significant findings when the various individual risk factors were examined by the three different odds ratios sub-typed by location (Table 3). Smoking as a continuous variable appeared to increase the risk of cancer for both proximal and distal cancers and in total. This mild effect remained for distal cancers with an OR₃ of 1.02 (95% CI: 1.00–1.03) but did not remain significant for proximal cancers. *H. pylori* infection was strongly associated with distal cancer risk with an OR₁ of 2.24 (95% CI: 1.23–4.09). However, for proximal cancers, *H. pylori* was a strong protective factor for all three ORs. The protective effect was statistically significant for both OR₂ and OR₃ (0.35 (95% CI: 0.17–0.72) and 0.38 (95% CI: 0.17–0.84), respectively). Fiber intake also resulted in a trend towards protective effects; for all cancers, the odds ratios exhibited a protective effect (OR₁=0.95, OR₂=0.95 and OR₃=0.89), with none of the 95% confidence intervals crossing unity. Increased fat consumption appeared to be a risk factor for distal tumors as the OR₃ was 1.03 (95% CI: 1.01–1.06).

3.3. Joint and component odds ratios

There appeared to be significant effects with smoking greater than 40 pack-years of cigarettes, high fat consumption, and low vitamin C intake (Table 3) when examining the main and joint effects for each covariate with the Pro/Pro polymorphism for all cancers. Tables 4 and 5 show the analysis of the main and joint effects separated by site (either proximal or distal stomach cancer).

There appeared to be a significant interaction between smoking and the Pro/Pro polymorphism for both OR₁ (3.55, 95% CI: 1.39–9.05) and OR₂ (2.76, 95% CI: 1.03–7.39)

for proximal cancers (Table 4). For OR₃, the point estimate was 1.96 but the confidence interval crossed one. Increased caloric intake appeared to have a statistical interaction with significant point estimates of 2.96 (95% CI: 1.00–6.54) for OR₁, and 3.54 (1.11–11.52) for OR₂. There also appeared to be an interaction with high fat intake as OR₁ had a significant point estimate of 2.56 (95% CI: 1.00–6.54), which did not remain significant when controlled for other risk factors.

For distal cancers (Table 5), there was a significant interaction between the Pro/Pro polymorphism and high fat consumption for OR₂ (3.29, 95% CI: 1.02–10.66) while OR₁ was nearly significant with an odds ratio of 2.70 (0.91–8.00). Controlling for all the confounders (OR₃) showed the same relationship with an odds ratio of 2.41, but the 95% CI was 0.53–10.92. Low vitamin C intake showed a very strong positive relationship with distal cancers: OR₁ was 4.82 (95% CI: 1.72–13.45), and OR₂ was 6.19 (95% CI: 2.08–18.40). OR₃ was nearly significant with a point estimate of 3.71 (95% CI: 0.99–13.98).

3.4. Interaction odds ratios

There were no significant effects by the interaction odds ratios for the interaction term with any of the risk factors when looking at all gastric cancers. The only significant interaction odds ratios by cancer site (proximal or distal) were for the proximal tumors; for high alcohol intake, OR₃ was 16.39 (95% CI: 1.63–165.13). The odds ratios for OR₁ and OR₂ were 2.88 and 3.77, but the 95% CIs crossed one. High sodium intake had an OR₃ of 14.22 (95% CI: 1.08–187.96), with OR₁ and OR₂ having odds ratios close to significance of 9.74 (95% CI: 0.98–97.09) and 9.51 (95% CI: 0.84–107.80), respectively. High caloric intake also had a significant OR₃, with a point estimate of 17.98 (1.27–254.17), with OR₁ and OR₂ showing similar trends with point estimates of 9.33 (95% CI: 0.93–93.58) and 10.56 (95% CI: 0.91–122.71), respectively. Both OR₁ and OR₂ for increased fiber consumption showed a significant protective interaction; with the Pro/Pro polymorphism as the point estimate, the ORs were 0.18 (95% CI: 0.03–0.96) and 0.14 (95% CI: 0.02–0.87). The nonsignificant point estimate for OR₃ (0.20) also showed the same protective trend. Interestingly, for proximal tumors, the interaction odds ratio could not be calculated because there were no cases with the Pro/Pro polymorphism with low fat consumption (there were 10 controls available).

4. Discussion

Methodological issues need to be discussed before our results can be interpreted. We studied the effects of p53 codon 72 polymorphisms for the development of gastric cancer first by first employing a case-control technique combining the results of all cancers and other potential risk factors. We then examined the data by location of cancer, which was classified as either proximal stomach/distal esophagus or distal stomach. Next we assessed any gene/environment interactions by looking at the joint and component effects of the high-risk (Pro/Pro) polymorphism (using a case-case design), while calculating the point estimate of the interaction term with other potential risk factors. We then assessed any type of dose escalation response by examining the effects of exposure by quartiles, or in the case of smoking and drinking, by examining the effects of increasing exposure by predetermined amounts. Ideally, any study evaluating the relationship of potential progression to cancer

would be prospective (either a prospective cohort study or a nested case-control study). However, such a study would probably not be feasible due to the slow development of the disease and because acquiring the disease is still a relatively rare event.

The major limitation of our study design is its retrospective nature: the collection of cases and controls and the acquisition of epidemiologic data were done at the time of enrollment into the study (prevalent cases) and were not done prospectively. This inherently leads to problems with temporality of association (which could lead to a false conclusion) and recall/reporting bias, which would lead to an overestimation of effect. In the case of reporting bias, subjects may preferentially underestimate or overestimate their exposure if certain habits or exposures were found to be socially and/or personally unacceptable, which could lead to either overestimation or underestimation of effect.

There is also a potential for misclassification of disease status or exposure status. In the case of the p53 codon 72 polymorphisms, many of these pitfalls are avoided; due to the fact these polymorphisms are genetically inherited and cannot be altered, problems with temporal ambiguity are not an issue (though for other exposures, such as with *H. pylori*, it remains an issue). As for misclassification, tests to determine genotype were done in a blinded fashion and repeated to confirm results. For potential misclassification of disease, as stated earlier, there was excellent correlation with the pathology reports to a random sampling of cases that were read by an independent pathologist. Recall bias and to some extent reporting bias may have been limited, since again the polymorphism is inherited and cannot be changed. Patients also would have no way of knowing their genetic status since this test was done many years after enrollment into this study. Also, patients were given the HHHQ prior to their upper endoscopy at MSKCC so that they did not know their cancer/control status prior to answering the questionnaire. However, since MSKCC is a major tertiary care referral-based hospital, it is possible that patients knew of their diagnosis by outside procedures prior to their tests at MSKCC, or that they were symptomatic prior to their procedure at MSKCC, thus leading subjects to infer that they had cancer.

Since this was a hospital-based case-control study, a major concern was the selection of subjects. In general, hospital-based controls may not be representative of the general population and can be affected by referral bias and/or selection bias. In order to reduce this bias, both cases and controls were selected from the same endoscopic unit. Another limitation of this study was low power and precision. Our study had 155 cases of gastric cancer and 134 cancer-free controls, but only 87 cardia/distal esophageal cases and 68 distal gastric cancers. For the interactions between the Pro/Pro polymorphism and various risk factors, many cells had a small number of cases ($n = 9$). This relatively small sample size limits our ability to estimate the effects precisely after controlling for several potential confounding factors, especially when examining the effects by site (subset analysis). Any positive or negative association results by site should be considered exploratory in nature. However, examining the univariate results of our data for risk of gastric cancer by site showed associations that were consistent with previously published data thus lending credibility to our data and the cohort of patients.

In this study we did not see any significant relationship between the Pro/Pro p53 codon 72 polymorphism and gastric cancer, either together or separated by site. When analyzed for any potential interactions with known risk factors for gastric cancer, there were a few significant associations. When analyzing by total cancers, there were significant joint effects with smoking ($OR_1=3.55$ (95% CI: 1.39–9.05), $OR_2=2.76$ (95% CI: 1.03–7.39)) and high fat consumption ($OR_1=2.61$ (95% CI: 1.13–6.06), $OR_2=2.85$ (95% CI: 1.14–7.15) (Table 3). Increased smoking and high fat consumption both displayed a positive interactive effect with smoking, displaying a more than additive effect (OR_1) and with high fat consumption showing a multiplicative effect (OR_1 and OR_2). However, neither had a significant interaction odds ratio (odds ratio = 0.87 (95% CI: 0.24–3.12) for smoking, 1.40 (95% CI: 0.37–5.29) for fat OR_1 , and 1.13 (95% CI: 0.27–4.70) for fat OR_2).

For proximal tumors, smoking ($OR_1=3.93$), high alcohol consumption ($OR_1=2.94$), high fat consumption ($OR_1=2.56$) and high caloric intake ($OR_1=2.96$, $OR_2=3.54$) all showed statistically significant joint effects with the Pro/Pro polymorphism. Increased alcohol consumption, high caloric intake and smoking exhibited a multiplicative effect. Fat intake most likely would have shown a more than multiplicative effect; however, there were no cases with both low fat consumption and the Pro/Pro polymorphism (Table 4). (Adding 0.5 to all the cells involved in calculating the odds ratio, the odds ratio for low fat consumption and the Pro/Pro polymorphism would be 0.10 (95% CI: 0.006–1.81), which would indicate a more than multiplicative negative effect based on the crude estimates). Analyzing the interaction odds ratios for alcohol consumption showed a trend with the point estimates ($OR_1=2.88$, $OR_2=3.77$, $OR_3=16.39$), but only OR_3 was significant, and the confidence intervals were extremely wide (95% CI: 1.63–165.13). The interaction odds ratios for high caloric intake also showed a similar trend, but again the confidence intervals were extremely large, indicating sparse data ($OR_1=9.33$ (95% CI: 0.98–97.09), $OR_2=10.56$ (95% CI: 0.91–122.71), $OR_3=14.22$ (95% CI: 1.27–254.17)). For high fat consumption [already mentioned above], no interaction odds ratios could be calculated because there was no case within that particular stratum. However, an interaction odds ratio for OR_1 was calculated to be 15.33 once 0.5 was added to all cells to prevent a value of zero in any of the cells. Very high point estimates for interaction odds ratios were also seen with high sodium intake. For OR_1 it was 9.74 (95% CI: 0.98–97.09); for OR_2 it was 9.54 (95% CI: 0.84–107.80) and for OR_3 it was 14.22 (95% CI: 1.08–187.96). Again, due to the sparse data within groups, the confidence intervals were extremely large and the interpretation of the significant and nonsignificant values must be interpreted with caution.

Also, only low vitamin C intake in distal tumours showed any interesting joint effects with the Pro/Pro polymorphism. The low vitamin C intake/high-risk polymorphism group (Pro/Pro) had an OR_1 of 4.82 (95% CI: 1.72–13.45). Adjusting for age, sex and education the OR_2 was 6.19 (95% CI: 2.08–18.40). The results indicated a more than multiplicative effect of low vitamin C intake and the Pro/Pro polymorphism. Looking at the interaction odds ratios, the point estimates were increased but not statistically significant ($OR_1=4.25$ (95% CI: 0.66–27.50), $OR_2=4.73$ (95% CI: 0.67–33.43), $OR_3=5.55$ (95% CI: 0.66–46.47)).

Though we demonstrated no increased gastric cancer risk either in total or by site with the p53 codon 72 polymorphisms, we did find some interesting associations. High fat intake and

high caloric intake showed some interesting associations with proximal esophageal/cardia tumors, with high fat intake associated with a statistically significant increased joint interaction ($OR_1=2.56$; 95% CI: 1.00–6.54). An interaction term odds ratio was calculated at 15.33 (done by adding 0.5 to all the cells to prevent a 0 value), and there appeared to be a significant increase in gastric cancer risk between the highest and lowest quartiles of consumption ($OR_1=2.23$; 95% CI: 1.12–4.42) with a p value for trend = 0.03 (data not shown). For high caloric intake, the joint effect was 2.96 (95% CI: 1.00–7.99) for OR_1 and 3.54 (95% CI: 1.11–11.32) for OR_2 (both with a more than multiplicative effect) with interaction ORs of 9.33 (95% CI: 0.93–93.58) and 10.56 (95% CI: 0.91–122.71), respectively. Data for high fat consumption has primarily been linked to breast, prostate and colon cancer, but more recently high fat consumption has been linked epidemiologically to cardia/distal esophageal adenocarcinoma. However, the exact mechanism for high fat intake leading to carcinogenesis in esophageal cancer has not yet been elucidated, thus making it difficult to explain why there would be an interaction with any p53 polymorphism. High calorie intake and esophageal/cardia cancer could be explained by a variety of factors. The first would be that a high-calorie diet would potentially be associated with a high-fat diet. Another potential reason would be that a high-calorie diet would be associated with obesity and/or subsequently hiatal hernias, which have both been linked to cardia/distal esophageal adenocarcinoma [28]. A third potential reason would be that increased caloric intake leads to increased cellular division and/or oxidative stress and subsequent increased risk of developing cellular damage. An attractive hypothesis would be that high caloric consumption leads to increased cellular proliferation/growth, and in conjunction with the high-risk Pro/Pro p53 polymorphism, would attenuate apoptosis and regulation of defective/damaged cells, in turn leading to an increased risk of cancer. Energy restriction has been well documented in the basic science literature to have profound inhibitory effects on carcinogenesis due to its effect on various growth factors, oncogenes and tumor suppresser genes. Energy restriction has been shown to decrease cell proliferation and increase apoptotic cell death as well as having specific effects on the cell cycle regulatory pathways [29].

Looking at distal cancers, low vitamin C intake also was associated with an increased joint risk and a more than multiplicative increase in the unadjusted and adjusted odds ratios. The point estimates for the interaction odds ratios were also increased, though the increase was not statistically significant. The role of vitamin C as a potential inhibitor of gastric cancer carcinogenesis has been widely documented. Epidemiologically, retrospective and prospective studies of vitamin C serum levels and dietary intake are consistent with a reduction in stomach cancer risk; 12 of 13 case-control studies have related increased dietary intake of vitamin C as resulting in a decreased risk of developing gastric cancer [30]. In prospective studies performed in China and Switzerland, high serum levels of vitamin C were associated with a decrease in stomach cancer and dysplasia [31,32]. In a recent randomized clinical trial in a high-risk area of Columbia, vitamin C supplementation was shown to decrease precancerous lesions [33]. Presumably, vitamin C, an important antioxidant, inhibits carcinogenesis by neutralizing reactive oxygen species that can damage DNA or by inhibiting nitrosamines [34–37]. In conjunction with the protective p53 polymorphism, one can easily postulate that some form of synergistic interaction could

potentially occur through their proposed mechanisms of action. However, any conclusions based on these findings must be tempered by the potential weakness of this study, which has been previously addressed. Further epidemiologic and basic science studies with larger groups need to be done to validate the results of this exploratory study, looking a priori at the relationship between this polymorphism and high fat intake, along with vitamin C intake, especially by cancer site (cardia/distal esophageal and distal cancers).

Acknowledgments

This work was supported in part by the Division of Digestive Diseases, David Geffen School of Medicine at UCLA, NIH Training Grant T32 DK07180 and the Alper Research Program for Environmental Genomics of the Jonsson Comprehensive Cancer Center Training Grant T32 CA09142. I would also like to acknowledge the STAR program at UCLA for their support in my fellowship training.

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Table 1

Demographic Characteristics and p53 Codon 72 Polymorphism Genotypes

	Controls No. (%)	Gastric Cancer (GC)			Total p1	Prox p2	Dist p3
		GE and Cardia No. (%)	Distal No. (%)	Total No. (%)			
Total	134	87	68	155			
Age							
<60	77 (57.5)	38 (43.7)	27 (39.7)	65 (41.9)	0.008	0.02	0.05
≥ 60	57 (42.5)	49 (56.3)	41 (60.3)	90 (58.1)			
Sex							
Male	68 (50.7)	73 (83.9)	43 (63.2)	116 (74.8)	0.001	0.001	0.09
Female	66 (49.3)	14 (16.1)	25 (36.8)	39 (25.2)			
Race							
White	111 (82.8)	82 (95.3)	52 (76.5)	134 (87.0)	0.32	0.006	0.28
Non-White	23 (17.2)	4 (4.7)	16 (23.5)	20 (13.0)			
Education							
< 12 years	46 (35.4)	40 (46.0)	24 (35.8)	64 (41.6)	0.29	0.12	0.95
≥ 12 years	84 (64.6)	47 (54.0)	43 (64.2)	90 (58.4)			
p53 Codon 72 Polymorphism							
Pro/Pro	22 (18.2)	16 (18.8)	15 (21.7)	31 (20.1)	0.70	0.32	0.16
Pro/Arg	61 (45.5)	41 (59.1)	32 (47.8)	73 (46.8)			
Arg/Arg	51 (36.3)	30 (22.1)	21 (30.5)	51 (33.1)			

p1 = P value of total cases vs. controls; p2 = P value of proximal cases vs. controls; p3 = P value of distal cases vs. controls.

Table 2

Odds Ratios (OR) and 95% Confidence Intervals (CI) of High Risk Genotype (Pro/Pro)

Controls (N = 134)		Gastric Cancer (GC)		
		GE and Cardia (N = 87)	Distal (N = 68)	Total (N = 155)
OR1 (Crude) and 95%CI	Reference 1.00	1.15 (0.56–2.33)	1.44 (0.69–3.00)	1.27 (0.70–2.33)
OR2 and 95%CI*		1.05 (0.47–2.36)	1.53 (0.71–3.29)	1.36 (0.71–2.60)
OR3 and 95%CI*		0.73 (0.27–1.97)	1.36 (0.57–3.25)	1.02 (0.49–2.14)

* OR1: crude odds ratio; OR2: odds ratio adjusted for age, sex, race, education; OR3: odds ratio adjusted for age, sex, race, education, BMI, Calories, Sodium, Smoking, Vitamin C, Fiber, Alcohol, Fat, *H. pylori*.

Table 3

Main and Joint Effects of Potential Risk Factors on Gastric Cancer Risk

Total vs. Control	Variable	Cases	Controls	OR1	(95% C.I.)	OR2	(95% C.I.)	OR3	(95% C.I.)
Pro/Pro genotype									
<i>Smoking</i>									
No	No	58	80	1.00		1.00		1.00	
No	Yes	65	28	3.20	1.83–5.59	2.40	1.32–4.38	2.01	0.99–4.05
Yes	No	13	14	1.28	0.56–2.93	1.40	0.58–3.39	1.06	0.40–2.80
Yes	Yes	18	7	3.55	1.39–9.05	2.76	1.03–7.39	1.96	0.65–5.93
Interaction OR		154	129	0.87	0.24–3.12	0.82	0.21–3.16	0.92	0.21–4.07
<i>Alcohol</i>									
No	No	51	52	1.00		1.00		1.00	
No	Yes	73	60	1.24	0.74–2.08	1.16	0.65–2.05	0.76	0.37–1.55
Yes	No	12	12	1.02	0.42–2.48	1.25	0.48–3.25	0.82	0.29–2.37
Yes	Yes	19	10	1.94	0.82–4.57	1.71	0.68–4.29	0.93	0.31–2.80
Interaction OR		155	134	1.53	0.45–5.20	1.19	0.32–4.41	1.49	0.33–6.65
<i>Sodium</i>									
No	Low	56	55	1.00		1.00		1.00	
No	High	56	49	1.12	0.66–1.92	1.10	0.61–1.99	0.82	0.33–2.01
Yes	Low	9	11	0.80	0.31–2.09	0.98	0.35–2.77	0.72	0.24–2.14
Yes	High	19	10	1.87	0.80–4.37	1.93	0.77–4.84	1.12	0.33–3.80
Interaction OR		140	125	2.07	0.57–7.47	1.77	0.44–7.10	1.90	0.42–8.56
<i>Fiber</i>									
No	High	42	53	1.00		1.00		1.00	
No	Low	70	51	1.73	1.01–2.98	1.95	1.08–3.52	2.06	0.93–4.57
Yes	High	15	10	1.89	0.77–4.64	1.96	0.74–5.20	1.42	0.47–4.31
Yes	Low	13	11	1.49	0.61–3.67	2.01	0.77–5.20	1.66	0.56–4.95
Interaction OR		140	125	0.46	0.13–1.60	0.53	0.14–2.04	0.57	0.13–2.46
<i>Fat</i>									
No	Low	38	52	1.00		1.00		1.00	
No	High	74	52	1.95	1.13–3.37	2.02	1.10–3.69	1.72	0.70–4.20

Total vs. Control	Variable	Cases	Controls	OR1	(95% C.I.)	OR2	(95% C.I.)	OR3	(95% C.I.)
Yes	Low	7	10	0.96	0.33–2.74	1.25	0.41–3.87	1.10	0.33–3.72
Yes	High	21	11	2.61	1.13–6.06	2.85	1.14–7.145	1.87	0.56–6.72
Interaction OR		142	125	1.40	0.37–5.29	1.13	0.27–4.70	0.99	0.21–4.63
<i>Calorie</i>									
No	Low	47	51	1.00		1.00		1.00	
No	High	65	53	1.33	0.78–2.28	1.37	0.75–2.51	1.11	0.42–2.94
Yes	Low	9	12	0.81	0.31–2.11	0.98	0.35–2.73	0.75	0.25–2.21
Yes	High	19	9	2.29	0.94–5.56	2.50	0.95–6.58	1.53	0.39–5.98
Interaction OR		140	125	2.12	0.58–7.68	1.87	0.47–7.45	1.85	0.42–8.23
<i>Vitamin C</i>									
No	High	53	50	1.00		1.00		1.00	
No	Low	59	54	1.03	0.60–1.76	1.29	0.72–2.32	0.97	0.43–2.15
Yes	High	8	12	0.63	0.24–1.67	0.64	0.23–1.83	0.44	0.13–1.48
Yes	Low	20	9	2.10	0.87–5.04	2.98	1.16–7.67	1.65	0.55–4.97
Interaction OR		140	125	3.23	0.88–11.93	3.60	0.88–14.69	3.91	0.81–18.75

Table 4

Main and Joint Effects of Potential Risk Factors on Proximal Gastric Cancer Risk

Proximal vs Control	Variable	Cases	Controls	OR1	(95% C.I.)	OR2	(95% C.I.)	OR3	(95% C.I.)
Pro/Pro genotype									
<i>Smoking</i>									
No	No	32	80	1.00		1.00		1.00	
No	Yes	39	28	3.48	1.85–6.57	2.62	1.28–5.34	1.94	0.82–4.57
Yes	No	5	14	0.89	0.30–2.68	1.03	0.31–3.48	0.68	0.17–2.72
Yes	Yes	11	7	3.93	1.40–11.03	2.27	0.73–7.09	1.41	0.36–5.56
Interaction OR									
		87	129	1.26	0.27–5.84	0.84	0.16–4.55	1.07	0.15–7.80
<i>Alcohol</i>									
No	No	23	52	1.00		1.00		1.00	
No	Yes	48	60	1.81	0.97–3.36	1.44	0.70–3.00	0.64	0.25–1.61
Yes	No	3	12	0.57	0.15–2.20	0.43	0.10–1.93	0.13	0.02–0.83
Yes	Yes	13	10	2.94	1.13–7.67	2.36	0.79–7.02	1.31	0.34–5.11
Interaction OR									
		87	134	2.88	0.56–14.71	3.77	0.61–23.43	16.39	1.63–165.13
<i>Sodium</i>									
No	Low	29	55	1.00		1.00		1.00	
No	High	35	49	1.36	0.73–2.53	1.48	0.71–3.07	1.01	0.32–3.18
Yes	Low	1	11	0.17	0.02–1.40	0.18	0.02–1.58	0.12	0.01–1.15
Yes	High	12	10	2.28	0.88–5.90	2.56	0.85–7.72	1.69	0.35–8.09
Interaction OR									
		77	125	9.74	0.98–97.09	9.51	0.84–107.80	14.22	1.08–187.96
<i>Fiber</i>									
No	Low	26	53	1.00		1.00		1.00	
No	High	38	51	1.52	0.81–2.85	1.55	0.75–3.21	2.24	0.83–6.01
Yes	Low	10	10	2.04	0.75–5.51	2.49	0.75–8.23	1.65	0.42–6.42
Yes	High	3	11	0.57	0.14–2.17	0.52	0.12–2.21	0.72	0.14–3.72
Interaction OR									
		77	125	0.18	0.03–0.96	0.14	0.02–0.87	0.20	0.03–1.47
<i>Fat</i>									
No	Low	24	52	1.00		1.00		1.00	
No	High	40	52	1.67	0.88–3.15	1.67	0.80–3.47	0.94	0.30–2.96

Proximal vs Control	Variable	Cases	Controls	ORI	(95% C.I.)	OR2	(95% C.I.)	OR3	(95% C.I.)
Yes	Low	0	10	0	0	0	0	0	0
Yes	High	13	11	2.56	1.00–6.54	2.56	0.87–7.58	1.21	0.28–5.28
Interaction OR		77	125	0.00	0.00	0.00	0.00	0.00	0.00
<i>Calorie</i>									
No	Low	23	51	1.00		1.00		1.00	
No	High	41	53	1.72	0.91–3.25	1.94	0.90–4.19	1.83	0.51–6.54
Yes	Low	1	12	0.19	0.02–1.51	0.17	0.02–1.55	0.12	0.01–1.21
Yes	High	12	9	2.96	1.09–7.99	3.54	1.11–11.32	3.79	0.65–22.10
Interaction OR		77	125	9.33	0.93–93.58	10.56	0.91–122.71	17.98	1.27–254.17
<i>Vitamin C</i>									
No	High	38	50	1.00		1.00		1.00	
No	Low	26	54	0.79	0.37–1.68	0.83	0.33–2.07	0.68	0.20–2.37
Yes	High	6	12	0.60	0.18–1.98	0.87	0.19–4.00	0.61	0.10–3.58
Yes	Low	7	9	0.97	0.28–3.41	0.97	0.23–4.14	0.74	0.14–3.84
Interaction OR		77	125	2.46	0.53–11.31	1.75	0.31–9.94	2.06	0.28–14.97

Table 5

Main and Joint Effects of Potential Risk Factors on Distal Gastric Cancer Risk

Distal vs Control	Variable	Cases	Controls	OR1	(95% C.I.)	OR2	(95% C.I.)	OR3	(95% C.I.)
<i>Pro/Pro genotype</i>									
<i>Smoking</i>									
No	No	26	80	1.00		1.00		1.00	
No	Yes	26	28	2.86	1.43–5.72	2.44	1.15–5.16	2.06	0.82–5.15
Yes	No	8	14	1.76	0.66–4.66	1.72	0.62–4.79	1.47	0.47–4.59
Yes	Yes	7	7	3.08	0.99–9.59	2.98	0.91–9.81	2.77	0.74–10.40
Interaction OR		67	129	0.61	0.13–2.82	0.71	0.15–3.50	0.91	0.16–5.35
<i>Alcohol</i>									
No	No	28	52	1.00		1.00		1.00	
No	Yes	25	60	0.77	0.40–1.49	0.80	0.40–1.61	0.79	0.32–1.95
Yes	No	9	12	1.39	0.52–3.71	1.69	0.61–4.70	1.68	0.53–5.31
Yes	Yes	6	10	1.11	0.37–3.39	1.04	0.32–3.33	0.71	0.17–2.98
Interaction OR		155	134	1.53	0.45–5.20	1.19	0.32–4.41	1.49	0.33–6.65
<i>Sodium</i>									
No	Low	27	55	1.00		1.00		1.00	
No	High	21	49	0.87	0.44–1.74	0.86	0.42–1.79	0.63	0.20–2.00
Yes	Low	8	11	1.48	0.53–4.11	1.55	0.53–4.52	1.30	0.41–4.16
Yes	High	7	10	1.43	0.49–4.16	1.47	0.47–4.60	0.90	0.20–4.06
Interaction OR		63	125	1.10	0.25–4.92	0.86	0.42–1.79	1.10	0.18–6.73
<i>Fiber</i>									
No	High	16	53	1.00		1.00		1.00	
No	Low	32	51	1.73	1.01–2.98	1.95	1.08–3.52	2.06	0.93–4.57
Yes	High	5	10	1.89	0.77–4.64	1.96	0.74–5.20	1.42	0.47–4.31
Yes	Low	10	11	1.49	0.61–3.67	2.01	0.77–5.20	1.66	0.56–4.95
Interaction OR		63	125	0.88	0.19–4.11	0.97	0.19–5.01	1.04	0.16–6.96
<i>Fat</i>									
No	Low	14	52	1.00		1.00		1.00	
No	High	34	52	2.43	1.17–5.05	2.74	1.25–6.02	2.48	0.78–7.87

Distal vs Control	Variable	Cases	Controls	OR1	(95% C.I.)	OR2	(95% C.I.)	OR3	(95% C.I.)
Yes	Low	7	10	2.60	0.84–8.06	2.79	0.84–9.21	2.37	0.64–8.77
Yes	High	8	11	2.70	0.91–8.00	3.29	1.02–10.66	2.41	0.53–10.92
Interaction OR		65	125	0.43	0.09–1.95	0.43	0.09–2.16	0.41	0.07–2.41
<i>Calorie</i>									
No	Low	24	51	1.00		1.00		1.00	
No	High	24	53	0.96	0.49–1.91	1.05	0.50–2.18	0.65	0.19–2.18
Yes	Low	8	12	1.42	0.51–3.92	1.54	0.53–4.47	1.27	0.39–4.15
Yes	High	7	9	1.65	0.55–4.97	1.83	0.57–5.93	0.84	0.16–4.51
Interaction OR		63	125	1.21	0.27–5.42	1.14	0.24–5.47	1.01	0.17–6.15
<i>Vitamin C</i>									
No	High	15	50	1.00		1.00		1.00	
No	Low	33	54	2.04	0.99–4.19	2.49	1.15–5.36	1.75	0.60–5.10
Yes	High	2	12	0.56	0.11–2.76	0.53	0.10–2.82	0.38	0.06–2.41
Yes	Low	13	9	4.82	1.72–13.45	6.19	2.08–18.40	3.71	0.99–13.98
Interaction OR		63	125	4.25	0.66–27.50	4.73	0.67–33.43	5.55	0.66–46.47