Research Article

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Aromatic DNA Adducts and Risk of Gastrointestinal Cancers: A Case–Cohort Study within the EPIC–Spain

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Abstract

Background: Colorectal (CRC) and gastric cancer (GC) are associated with meat intake and tobacco smoke, maybe because of aromatic compounds occurring in tobacco smoking and formed during cooking meat. Activated metabolites of these compounds may bind to DNA forming bulky adducts.

Methods: Forty-eight subjects diagnosed of GC and 154 of CRC during a 7-year follow-up period in the European Prospective Investigation into Cancer and Nutrition–Spain cohort were compared with a sample of 296 subjects using a case–cohort approach. Aromatic adducts to DNA from leukocytes collected at recruitment were measured by means of the ^{32}P-postlabeling technique. The relative risk (RR) and 95% confidence interval (CI), adjusted by relevant confounders were estimated by a modified version of Cox regression.

Results: Using the $\log_2$-transformed adduct concentration, we observed a RR = 1.57 (CI: 1.25–1.97) for CRC, which means a 57% increased risk associated with doubling the level of adducts, and 47% (RR = 1.47, CI: 1.07–2.00) increase in risk of GC. The association was more marked for colon than for rectal tumors.

Conclusions: The level of aromatic adducts in the DNA is independently associated with an increased risk of gastric and CRCs. This effect could be due to aromatic compounds present in tobacco smoke or formed in meat, but they could be also due to genotoxic compounds from other sources.

Impact: Sources of aromatic compounds should be taken into account, in addition to known risk factors, in the research and prevention of tumors of the stomach, colon, and rectum. *Cancer Epidemiol Biomarkers Prev; 21(4); 685–92. ©2012 AACR.*

Introduction

Colorectal cancer (CRC) is the third most common cancer in men and the second in women. Worldwide, 1.2 million new CRC cases and 609,000 deaths were expected to occur in 2008 (1). Gastric cancer (GC) is the fourth most commonly diagnosed cancer and the second leading cause of cancer death worldwide, with an estimated 990,000 new cases and 738,000 cancer deaths the same year (1).

Both tumors are multifactorial diseases and may result from a combination of genetic susceptibility and environmental exposures, including tobacco smoke and dietary factors. The International Agency for Research on Cancer has included the stomach, colon, and rectum among the tumor sites causally associated with tobacco smoking (2, 3). With regard to dietary factors, although the association between meat and CRC is well established, the evidence for GC is less conclusive. A recent meta-analysis of prospective studies has shown that red and processed meat intake was associated with an increased risk of CRC, with similar results for colon and rectal cancer risk (4). Another meta-analysis reported that increased consumption of processed meat was associated with an increased risk of GC, though the possibility that this association was confounded or modified by other factors could not be ruled out (5). The risk of GC associated with meat consumption was also investigated in 2 large cohort study; one of them (6) found that red and processed meat intakes were associated with an increased risk of gastric

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noncardia cancer, whereas the other (7) did not find any association of red or processed meat with GC.

Several plausible biologic mechanisms have been suggested to explain the association of red and processed meats with cancer. Cooking meat at high temperatures may produce potential mutagenic compounds such as polycyclic aromatic hydrocarbons (PAH) and heterocyclic amines (HA; ref. 4); on the other hand, both PAH and HA are among the many potential carcinogens found in tobacco smoke (8). DNA adducts are commonly measured biomarkers of genotoxicity; their presence is used as measure of the biologically effective dose of genotoxic compounds bound to DNA as their target for carcinogenesis. Bulky adducts are formed by several groups of aromatic compounds, mainly the ubiquitous PAH (9, 10). The ^{32}P-postlabeling DNA technique has become one of the most popular tools for detecting bulky DNA adducts, as it requires small amount of DNA, is highly sensitive, and is applicable to a wide variety of DNA adduct structures (8, 9).

Actually bulky DNA adducts are markers both of exposure to genotoxic aromatic compounds and of the ability of the individual to metabolically activate carcinogens and to repair DNA damage. A meta-analysis including 3 prospective studies and 6 case–control studies analyzed the cancer risk associated with bulky DNA adducts measured in blood samples from subjects (11); a weakly statistically significant increase in risk was reported for lung cancer and bladder cancer, evident only in current smokers. So far no analytical studies have been published on the association of aromatic adducts with tumors of the stomach or the large bowel. However, increased levels of leukocyte PAH–DNA adducts have been found associated with higher risk of colorectal adenomas (12), and increased levels of bulky DNA adducts measured by ^{32}P-postlabeling have been detected in the colonic mucosa of colon adenocarcinoma patients (13), as well as in tumor tissue from patients undergoing surgery for GC (14). We aimed to assess the potential association between levels of aromatic DNA adducts and the risk of cancer of the stomach, colon, and rectum in Spanish adults.

Methods

Study design: Setting, participants, and data collection

The study subjects were participants from the Spanish cohort of the European Prospective Investigation into Cancer and Nutrition (EPIC), following a case–cohort design. The subcohort (referent group) were 300 subjects randomly selected, according to the age–sex structure of Spanish population, among the 41,438 healthy volunteers of the EPIC–Spain cohort, recruited between 1992 and 1996 in 5 regions of Spain; further details are provided elsewhere (15, 16). Case ascertainment was based on record-linkage with population cancer registries of the 5 regions where the cohort was recruited. Cases were defined as first occurrence of a primary tumor of the stomach (C16 of the ICD-10) or the colon or rectum (C18-20); all subjects newly diagnosed of these 2 tumor sites during the 7-year follow-up (49 GC and 156 CCR) were included as cases of the study.

Information on usual diet and lifestyle factors, anthropometry, and a 30-mL blood sample were obtained at recruitment. Usual food intake during the preceding year, taking into account seasonal variations, was estimated through a personal interview with a validated version of a dietary history questionnaire. The lifestyle questionnaire included questions on education, history of previous illnesses, lifetime history of tobacco smoking, and physical activity. Anthropometric measurements, including height, weight, and waist circumference were taken by trained persons; measured height and weight were then used to compute the body mass index (BMI) as kg/m^2. Blood samples were divided into 0.5-mL aliquots of plasma, serum, red blood cells, and buffy coat, and stored in liquid nitrogen at −196°C. Biologic material was not available for 4 subjects of the subcohort and 3 cases; thus the study population is composed of 296 controls, 48 GC cases, and 154 CCR cases (3 of which belonged to the subcohort).

DNA adducts determination

DNA from white blood cells (WBCs) was extracted from 100 to 150 μL buffy coats, using a method requiring enzymatic digestion of RNA and proteins followed by phenol–chloroform extraction; coded DNA was stored at −80°C until laboratory analysis. Aromatic DNA adducts were analyzed blindly using the nuclease P1 modification of the ^{32}P-DNA postlabeling technique; details of the technique have been previously reported (17, 18). The levels of DNA adducts were expressed as relative adduct labeling screen response in adducted nucleotides as compared with total nucleotides. The detection limit was 0.1 adduct per 10^9 normal nucleotides as reported (15).

Statistical methods

The levels of DNA adducts were expressed as adducted nucleotides per 10^9; samples below the detection limit were assigned a value of 0.05×10^{-9} (half the threshold of detection of the technique). Because the distribution of adducts was right-skewed, this variable was $\log_2$-transformed, after adding a constant of 1 to avoid the strong influence of negative values (in the log-scale) of subjects whose original adduct levels were very close to zero.

The association between the level of DNA adducts and CRC or GC was estimated by the relative risk (RR) and 95% confidence intervals (CI), based on the proportional hazards model (Cox regression) with a modified partial likelihood (19). Adjustments of the partial likelihood are required as the cases are overrepresented in the case–cohort set and therefore unadjusted risk sets in the partial likelihood would not represent the original cohort. Different weighting methods have been proposed to estimate this pseudo-likelihood, but when the subcohort is small, the Prentice's method seems to provide the estimates that most resemble to those from the full cohort (20).

Three different models were built; first, a minimally adjusted model included as covariates sex, age, and center, to take into account the stratified design of the subcohort, as well as season of blood extraction, as this variable had been found associated with the level of adducts (16). The adjusted model also included as covariates a list of potential confounders, including the main potential risk factors of the 2 types of cancers under study: for GC the model included education, energy, alcohol consumption, and intake of vegetables, fruits, fiber, and vitamin C; for CRC the model included education, physical activity, BMI, waist circumference, height, energy, alcohol consumption, and intake of vegetables, fruits, fiber, calcium, and folic acid. The fully adjusted model, in addition to the previous list, included as covariates the smoking status and the intake of red meat and processed meat, assumed to be the main sources of compounds that may produce aromatic adducts. Separate analyses were carried out for GC and CRC; for the latter we also explored the effect of adducts according to the localization of the tumor in the colon or rectum. Finally, we explored the potential effect modification of sex, smoking status, and the level of intake of fresh and processed meat, and fiber. Interaction between these variables and the level of aromatic adducts was assessed by the likelihood ratio test (21).

Results

The 154 CRC and 48 GC cases diagnosed in the EPIC–Spain cohort during a 7-year follow-up were compared with a subcohort of 296 subjects; the main features of the subcohort and both case series are shown in Table 1. There was a predominance of males among cases, but the differences were not statistically significant. Cases were significantly older than the subcohort members; the average ages at recruitment were 54.6 years for CRC cases, 54.5 years for GC cases, and 49.1 years for the subcohort. Cases also consumed more alcohol than controls: 21.4 and 19.5 grams/d for CRC and GC cases, respectively, as compared with 16.6 in the subcohort, but the difference was statistically significant for CRC only. No significant differences were observed for other demographic, lifestyle, or dietary factors.

The measured concentrations of aromatic DNA adducts were higher in both series of cases than in the subcohort (Fig. 1). The subcohort members had in average 5.9 adducts per 10^9 normal nucleotides (geometric mean), for 8.39×10^{-9} and 7.98×10^{-9} for GC and CRC cases, respectively. For both tumor sites the differences, adjusted for sex, age, center, and season of blood extraction, were statistically significant. Within the CRC cases, those located in the colon tended to have a higher adduct level than those located in the rectum (geometric means 8.11 vs. 7.63×10^{-9}), although the difference between them was not significant ($P = 0.62$).

The RRs of CRC and GC associated with increasing levels of adducts are shown in Table 2, using a categorized and a continuous version of the variable, and considering different levels of adjustment. In the minimally adjusted model, subjects in the second and third tertiles had slightly more than twice the risk of CRC, as compared with those in the lowest tertile (reference category). The corresponding RR for the continuous variable ($\log_2$-tranformed) was 1.50 (CI: 1.24–1.82), meaning that the risk of CRC increases by 50% for doubling the concentration of aromatic adducts. With regard to GC, the RR for the continuous variable was also 1.50 (CI: 1.09–2.07), though the RR for the tertiles were not significant given the small number of cases. Adjustment for the main confounders did not have any substantial influence on the association; for CRC the RR for the continuous variable slightly increased to 1.53, whereas for GC it reduced to 1.46; further inclusion of potential sources of aromatic adducts did not materially modify the estimates, with RR = 1.57 and RR = 1.47 for CRC and GC, respectively. In all these cases the estimates were statistically significant, with very similar CI. The association was more marked for colon than for rectal tumors; for instance, the fully adjusted RR were 1.75 (1.34–2.28) and 1.44 (0.99–2.09) for tumors located in the colon and the rectum, respectively.

We explored the potential effect modification of some factors on the association between aromatic adducts and CRC or GC (Table 3). No significant differences were seen for these associations between men and women. The risk of CRC associated with a higher level of adducts was more marked for the current than for never or former smokers, although the differences were not statistically significant (P value for interaction = 0.16). There was no differential effect of adducts on GC risk according to smoking status. The association between the adduct level and both CRC and GC risk tended to be higher among subjects with daily intake of red meat below the median, and the opposite was observed for processed meat; a higher risk of GC was also observed among those with higher consumption of fiber. However, no significant interactions were detected between the adduct levels and any of the dietary factors considered.

Although subgroup analysis is difficult for GC owing to the small number of cases, we examined the potential differential effect of the adduct level according to the localization and histology of the tumor. The localization of GC was available for 41 of the 48 cases, the majority of which were located in the distal (noncardia) region of the stomach. The fully adjusted RR for the $\log_2$-concentration of adduct was 1.34 (CI: 1.06–1.70). Among the 33 GC cases with available histology, 18 were of intestinal type and 15 diffuse; the respective RR for the continuous variable were 1.45 (0.91–2.31) and 1.34 (0.94–1.91); the difference between these 2 estimates was not statistically significant (P value 0.60). Finally, as diet could be modified by the early phases of the disease, the main analyses were carried out excluding the cases diagnosed during the first 2 years of follow-up (7 GC and 21 CRC). The main results remained minimally modified; for instance, the adjusted RR for the $\log_2$-transformed concentration of adducts were 1.57 (1.13–2.18) for GC and 1.61 (1.27–2.05) for CRC, whereas the fully adjusted RR were 1.57 (1.13–2.19) and 1.66 (1.28–2.16) for GC and CRC, respectively.

Table 1. Description of the main characteristics of the subcohort sample and case subjects (CRC and GC)

	Subcohort ($N = 296$)		CRC ($N = 154$)		GC ($N = 48$)	
	N (%)		*N* (%)		*N* (%)	
Sex						
Males	147 (49.7)		85 (55.2)		27 (56.3)	
Females	149 (50.3)		69 (44.8)		21 (43.8)	
Age group						
35–44 y	118 (39.9)		20 (13.0)		6 (12.5)	
45–54 y	99 (33.4)		51 (33.1)		15 (31.2)	
55–64 y	79 (26.7)		79 (51.3)		25 (52.1)	
>64 y	—		4 (2.6)		2 (4.2)	
Center						
Asturias	58 (19.6)		20 (13.0)		10 (20.8)	
Granada	59 (19.9)		21 (13.6)		9 (18.8)	
Guipúzcoa	60 (20.3)		52 (33.8)		6 (12.5)	
Murcia	59 (19.9)		33 (21.4)		5 (10.4)	
Navarra	60 (20.3)		28 (18.2)		18 (37.5)	
BMI						
<25 kg/m^2	67 (22.6)		24 (15.6)		7 (14.6)	
≥25 to <30 kg/m^2	147 (49.7)		77 (50.0)		27 (56.2)	
≥30 kg/m^2	82 (27.7)		53 (34.4)		14 (29.2)	
Smoking status						
Never smoker	174 (58.8)		78 (50.7)		25 (52.1)	
Former smoker	48 (16.2)		31 (20.1)		10 (20.8)	
Current smoker	74 (25.0)		45 (29.2)		13 (27.1)	
Education (highest school level)						
None	80 (27.3)		63 (41.5)		17 (35.4)	
Primary completed	124 (42.3)		54 (35.5)		20 (41.7)	
Technical/professional	33 (11.3)		11 (7.2)		6 (12.5)	
Secondary school	18 (6.1)		13 (8.6)		1 (2.1)	
University degree	38 (13.0)		11 (7.2)		4 (8.3)	
Physical activity						
Inactive	46 (15.5)		21 (13.6)		2 (4.2)	
Moderately inactive	56 (18.9)		42 (27.3)		13 (27.1)	
Moderately active	160 (54.1)		77 (50.0)		28 (58.3)	
Active	34 (11.5)		14 (9.1)		5 (10.4)	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
Dietary variables (daily intake)						
Energy (kcal)	2,238	751	2,313	783	2,139	773
Vegetables (g)	270.1	154	247.7	145	238.7	149
Fruits (g)	339.8	258	332.5	262	338.3	245
Red meat (g)	42.4	35.1	46.5	38.0	47.8	41.1
Processed meat (g)	44.1	43.0	41.4	67.0	34.5	34.1
Total meat (g)	137.4	72.2	142.1	67.0	125.4	57.7
Alcohol (g)	16.6	25.9	21.4	26.3	19.5	26.8
Fiber (g)	28.3	10.3	28.2	10.1	26.3	10.2
Calcium (mg)	910.2	364	883.7	435	819.8	353
Vitamin C (mg)	168.1	89.3	165.0	94.7	149.3	76.3
Folic acid (μg)	353.3	124	354.3	126	324.9	115

NOTE: Three subjects from the subcohort and 2 cases of CRC with missing information for educational level.
All the comparison (CRC cases with the subcohort and GC with the subcohort) are no significant ($P > 0.05$) except: age ($P < 0.0001$ both for CRC vs. subcohort and GC vs. subcohort), center (CRC vs. subcohort $P = 0.01$), and alcohol consumption (CRC vs. subcohort $P = 0.009$).

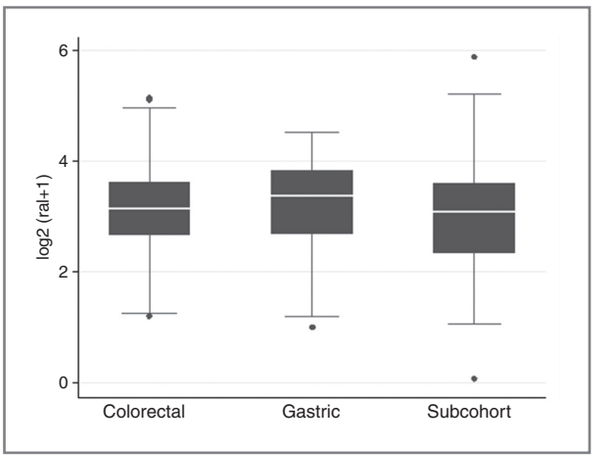

Figure 1. Box plot of DNA adducts in the subcohort sample and case subjects of colorectal and gastric cancer. RAL: relative adduct labeling (adducts per 10^9 normal nucleotides).

Discussion

We observed that the level of aromatic adducts in the DNA of WBCs was associated with an increased risk of cancer of the stomach, colon, and rectum. According to our study, doubling the concentration of adducts increased by 57% the risk of CRC and by 47% the risk of GC. This effect seems to be higher, though no significantly different, for tumors of the colon than for those located in the rectum. These associations seem not to be confounded by the main risk factors of CRC or GC.

As mentioned above, there are no previous results available assessing the effect of aromatic adducts in DNA from WBCs and tumors of the gastrointestinal tract (GI) tract. Indirect evidence comes from the association of potential sources of compounds originating these adducts, such as tobacco smoking (2, 3) and meat intake (4–7). A summary of the findings for the risk of cancer associated with meat and meat-related compounds (22) concluded that the evidence that red meat and processed meat are a cause of CRC is convincing, but only limited, though suggestive, for processed meat and GC. Even though this report considered the potential role of compounds formed in meat during cooking, such as PAH and HA, the overall judgment referred to the food (meat), not to any specific compound.

Further indirect evidence linking DNA adducts with tumors from the GI tract comes from studies assessing the presence of adducts in the gastric or colonic mucosa. With regard to GC, adducts measured by the ^{32}P-postlabeling were detected in all samples from tumor tissue of patients with GC (14); adduct levels were significantly greater in male subjects, and in the DNA of smokers than in that of non smokers. However, in

Table 2. RR for CRC and GC according to the level of aromatic DNA adducts

	RR (95% CI)			
	First tertile (reference)	Second tertile	Third tertile	Continuous (log_2)
Minimally adjusted model[a]				
CRC	1	2.12 (1.19–3.78)	2.15 (1.16–4.01)	1.50 (1.24–1.82)
Colon	1	2.06 (1.06–4.00)	2.46 (1.21–4.99)	1.60 (1.28–2.00)
Rectum	1	2.30 (0.95–5.58)	1.61 (0.60–4.31)	1.30 (0.99–1.71)
GC	1	1.47 (0.60–3.61)	2.52 (0.98–6.47)	1.50 (1.09–2.07)
Adjusted model[b]				
CRC	1	2.37 (1.23–4.55)	2.08 (1.04–4.15)	1.53 (1.23–1.89)
Colon	1	2.24 (1.06–4.72)	2.39 (1.10–5.17)	1.59 (1.25–2.03)
Rectum	1	3.15 (1.06–9.37)	1.58 (0.46–5.46)	1.44 (1.01–2.06)
GC	1	1.36 (0.51–3.64)	2.16 (0.79–5.88)	1.46 (1.08–1.97)
Fully adjusted model[c]				
CRC	1	2.30 (1.17–4.52)	2.29 (1.05–5.00)	1.57 (1.25–1.97)
Colon	1	2.38 (1.05–5.36)	3.24 (1.29–8.14)	1.75 (1.34–2.28)
Rectum	1	3.11 (1.05–9.27)	1.39 (0.33–5.88)	1.44 (0.99–2.09)
GC	1	1.39 (0.52–3.69)	2.18 (0.73–6.50)	1.47 (1.07–2.00)

NOTE: All the variables included in the model as they are shown in Table 1.

[a]Adjusted only for sex, age, center, and season of blood extraction.

[b]Adjusted for sex, age, center, season of blood extraction, education, energy, alcohol consumption, and intake of vegetables, fruits, fiber, and vitamin C (GC); adjusted for adjusted for sex, age, center, season of blood extraction, education, physical activity, BMI, waist circumference, height, energy, alcohol consumption, and intake of vegetables, fruits, fiber, calcium, and folic acid (CRC).

[c]Adjusted as previous model[(b)] plus smoking status, and intake of red meat and processed meat.

Table 3. RR for CRC and GC according to the level of aromatic DNA adducts by sex, smoking status, meat intake, and dietary fiber intake

	CRC[a]		GC[b]	
	Cases/ subcohort	RR (95% CI) for $\log_2$ (adducts/10^9 normal nucleotides)	Cases/ subcohort	RR (95% CI) for $\log_2$ (Adducts/10^9 normal nucleotides)
Sex				
Men	85/147	1.48 (1.14–1.93)	27/147	1.49 (1.00–2.22)
Women	69/149	1.71 (1.20–2.44)	21/149	1.52 (0.96–2.39)
P value for interaction		0.50		0.96
Smoking status				
Never	78/174	1.55 (1.17–2.05)	25/174	1.47 (1.03–2.08)
Former	31/48	1.32 (0.89–1.95)	23/122	1.59 (0.93–2.70)
Current	45/74	2.13 (1.14–3.98)		
P value for interaction		0.16		0.80
Red meat (grams/d)				
≤35.4	74/148	1.81 (1.35–2.43)	21/148	1.69 (1.14–2.51)
>35.4	80/148	1.26 (0.93–1.70)	27/148	1.31 (0.83–2.06)
P value for interaction		0.08		0.39
Processed meat (grams/d)				
≤32.6	82/148	1.40 (1.05–1.87)	27/148	1.36 (0.92–2.00)
>32.6	72/148	1.70 (1.25–2.30)	21/148	1.67 (1.05–2.66)
P value for interaction		0.35		0.46
Fiber (grams/d)				
≤26.93	73/148	1.63 (1.19–2.25)	27/148	1.24 (0.88–1.73)
>26.93	81/148	1.50 (1.11–2.02)	21/148	1.93 (1.09–3.41)
P value for interaction		0.69		0.16

[a]Adjusted for sex, age, center, season of blood extraction, education, physical activity, BMI, waist circumference, height, energy, alcohol consumption, smoking status, and intake of vegetables, fruits, fiber, calcium, folic acid, red meat, and processed meat.
[b]Adjusted only for sex, age, center, and season of blood extraction; adjustment for other factors was not possible owing to the small number of GC cases. Moreover, former and current smokers were combined into a single category also because of small number of cases.

another study there was no statistically significant difference in adduct levels between subjects with normal mucosa and those from patients with chronic atrophic gastritis and intestinal metaplasia (23). A recent study analyzed DNA adducts in the mucosa and adjacent muscle layer of the nontumoral part of stomach from patients with gastric neoplasms (24); mucosa-specific DNA adducts were found in all samples but were entirely absent from the adjacent muscle layers, suggesting that the gastric mucosa was exposed to DNA-reactive substances. With a different approach, specific benzopyrene-diol-epoxyde (BPDE) adducts were measured by ELISA assay in tumor and tumor-adjacent tissues of GC patients as well as in normal stomach tissues (25). Higher levels of total BPDE adduct were observed in tumor and nontumor tissues from subjects with GC than in normal stomach tissues, but the differences were not significant. With regard to CRC, ^{32}P-postlabeling analysis of DNA from tumoral mucosa of CRC patients showed significantly higher adduct levels than in DNA from colonic mucosa of patients without CRC (13); however, the adduct levels of tumoral and nontumoral mucosa of the same patients did not show significant differences. DNA of normal mucosa and the adjacent muscular layer from colorectal neoplasms was examined by ^{32}P-postlabeling analysis (26); although several common spots were present in the mucosa, there was no muscular layer–specific DNA adduct. DNA adduct levels were investigated in patients with CRC (tumoral and nontumoral tissues) and control patients (27); the adduct level was significantly higher in nontumoral than in control or tumoral colon samples. Human colonic biopsies were collected from healthy controls, polyp patients, and colon cancer patients (28); there were no significant differences in the total levels of DNA adducts between any of the groups. High-performance liquid chromatography (HPLC) after ^{32}P-postlabeling analysis suggested that some of these adducts could correspond to one HA, but with similar levels in tissues from controls,

polyp patients, or cancer patients. HPLC fluorescence was also applied to human colon mucosa samples where BPDE bound to DNA was detected (29), suggesting that benzo[a]pyrene and other PAH could play a role in the etiology of human CRC.

Among the strengths of our study it is worthy to note its prospective design and a good control of confounding. Because the blood samples were collected years before GC or CRC diagnosis, the disease itself could not have any influence in the exposure marker. With regard to potential confounders, we included as covariates most relevant risk factors of CRC and GC, although actually most of them were not associated with adduct levels in our population as previously reported (16). Among the main determinants of CRC or GC, only the infection by *Helicobacter pylori*, a major risk factor of GC, was not accounted for. Serology of *H. pylori* was available for 43 of the 48 GC cases, being positive for 41 of them, but was not available for the subcohort. However, a recent study (30) suggests that *H. pylori* infection is a necessary condition for gastric carcinogenesis and, therefore, adjustment or stratified analysis by *H. pylori* would be uninformative, at least for noncardia GC. In addition to potential confounders we also investigated the effect according to levels of the potential sources of DNA adducts. In our study, neither red meat nor processed meat were associated with CRC or GC; with regard to tobacco smoking, the RR for ever smokers were 2.34 (CI: 1.24–4.48) for CRC and 1.33 (0.59–2.92) for GC. In the stratified analysis there seems to be a higher effect of adducts on CRC among lower consumers of meat, as well as among current smokers, though the latter was no significant, and there was no differential effect on the risk of GC. This suggests that either aromatic adducts from other sources are relevant for the risk of CRC or GC, and/or that the effect of tobacco smoking and meat intake on CRC and GC may be also because of other compounds present in meat and tobacco smoke. We further explored the association of adducts with GC or CRC by levels of fiber intake as previous studies had suggested that dietary fiber was independently associated with the level of adducts (31), but no effect modification was observed. Furthermore, as already mentioned, adducts are markers of both exposure to and metabolism of potential carcinogens; thus measurement of adducts might be a better assessment of the transformed compounds from numerous PAH and HA from different sources, which may be difficult to assess by means of traditional questionnaires.

Among the limitations of our study one must take into consideration limited sample size, as well as those attributable to using aromatic adducts measured by ^{32}P-postlabeling in DNA from WBCs as markers of exposure. Ours is a relative small study, but statistical power is not an issue, at least for the main objective of the study, as we actually found significant associations. Of course limited sample size prevents subgroup analysis for GC. Another issue is the time frame of the exposure: we measured adducts in DNA extracted from buffy coat including a mixture of different types of WBCs, including 3 different subpopulations: granulocytes, monocytes, and lymphocytes. The lifespan of lymphocytes varies from a few days to several years making these cells potentially useful in the determination of long-term exposure, but the half-life of monocytes and granulocytes is much shorter, of hours or days, representing only recent exposure (9). On the other hand, WBCs are a surrogate tissue, not the target tissue for the disease in question. The primary rationale for the use of surrogate tissues is that they can be sampled by less invasive means, but the assumption that adduct levels measured in WBCs are a surrogate for those at the target tissue may not be valid: some studies that assessed adduct levels in target and surrogate tissues from the same individuals found strong correlations, whereas others have not (32). Finally, another limitation in the interpretation of our results refer to the actual composition of bulky adducts, as the ^{32}P-postlabeling is unable to determine the structure of the labeled adducts. The nuclease P1 version of the ^{32}P-postlabeling technique is generally used to analyze the formation of adducts induced by PAH, but this assay is also effective for the detection of DNA adducts induced from different hydroxyaryl amines, including aromatic amines resistant to the 3′-dephosphorylating action of nuclease P1 (18).

To conclude, the level of aromatic adducts in the DNA of WBCs is independently associated with an increased risk of gastric and CRCs; for the latter this effect is evident for tumors of the colon as well for those located in the rectum. This effect could be due to PAH or other aromatic compounds present in tobacco smoke or formed in meat, but they could be also due to genotoxic compounds from other sources.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: A. Agudo, M. Peluso, M.-J. Sanchez, C.A. González.
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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, and computational analysis): A. Agudo, A. Munia, L. Luján-Barroso, M.-J. Sanchez, N. Sala, C.A. González.
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