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RESEARCH PAPER

Dietary inflammatory index and inflammatory gene interactions in relation to colorectal cancer risk in the Bellvitge colorectal cancer case–control study

Raul Zamora-Ros · Nitin Shivappa · Susan E. Steck · Federico Canzian · Stefano Landi · M. Henar Alonso · James R. Hébert · Victor Moreno

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Abstract Chronic inflammation is an important factor in colorectal carcinogenesis. However, evidence on the effect of pro-inflammatory and anti-inflammatory foods and nutrients is scarce. Moreover, there are few studies focusing on diet–gene interactions on inflammation and colorectal cancer (CRC). This study was designed to investigate the association between the novel dietary inflammatory index (DII) and CRC and its potential interaction with polymorphisms in inflammatory genes. Data from the Bellvitge Colorectal Cancer Study, a case–control study (424 cases with incident colorectal cancer and 401 hospital-based controls), were used. The DII score for each participant was obtained by multiplying intakes of dietary components from a validated dietary history questionnaire by literature-based dietary inflammatory weights that reflected the inflammatory potential of components. Data from four important single nucleotide polymorphisms located in genes thought to be important in inflammation-associated CRC: i.e., interleukin (*IL*)-*4*, *IL-6*, *IL-8*, and peroxisome proliferator-activated receptor-γ (*PPARG*) were analyzed. A direct association was observed between DII score and CRC risk ($OR_{Q4\ vs.\ Q1}$ 1.65, 95 % CI 1.05–2.60, and *P* trend 0.011). A stronger association was found with colon cancer risk ($OR_{Q4\ vs.\ Q1}$ 2.24, 95 % CI 1.33–3.77, and *P* trend 0.002) than rectal cancer risk ($OR_{Q4\ vs.\ Q1}$ 1.12, 95 % CI 0.61–2.06, and *P* trend 0.37). DII score was inversely correlated with SNP rs2243250 in *IL-4* among controls, and an interaction was observed with CRC risk. Neither correlation nor interaction was detected for other inflammatory genes. Overall, high-DII diets are associated with increased risk of CRC, particularly for colon cancer, suggesting that dietary-mediated inflammation plays an important role in colorectal carcinogenesis.

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R. Zamora-Ros
Biomarkers Group, Nutrition and Metabolism Section, International Agency for Research on Cancer (IARC), Lyon, France

N. Shivappa · S. E. Steck · J. R. Hébert
South Carolina Statewide Cancer Prevention and Control Program, University of South Carolina, Columbia, SC, USA

N. Shivappa · S. E. Steck · J. R. Hébert
Department of Epidemiology and Biostatistics, Arnold School of Public Health, University of South Carolina, Columbia, SC, USA

F. Canzian
Genomic Epidemiology Group, German Cancer Research Center (DKFZ), Heidelberg, Germany

S. Landi
Department of Biology, University of Pisa, Pisa, Italy

M. H. Alonso · V. Moreno (✉)
Cancer Prevention and Control Program, Catalan Institute of Oncology (ICO) and CIBERESP, Barcelona, Spain
e-mail: v.moreno@iconcologia.net

M. H. Alonso · V. Moreno
Bellvitge Biomedical Research Institute (IDIBELL), Barcelona, Spain

V. Moreno
Department of Clinical Sciences, Faculty of Medicine, University of Barcelona, Barcelona, Spain

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Abbreviations

CI	Confidence interval
CRC	Colorectal cancer
CRP	C-reactive protein
DII	Dietary inflammatory index
IL	Interleukin
OR	Odds ratio
PPARG	Peroxisome proliferator-activated receptor-γ
SNPs	Single nucleotide polymorphisms

Introduction

Colorectal cancer (CRC) is the third most frequently occurring cancer and the fourth most common cause of death from cancer worldwide (Ferlay et al. 2013). A considerable body of evidence suggests that inflammation plays a key role in the pathogenesis of CRC by stimulating angiogenesis, damaging DNA, and chronically stimulating cell proliferation (Coussens and Werb 2002). Thus, patients with a history of chronic inflammatory bowel diseases have an increased risk of developing CRC (Laukoetter et al. 2011), whereas habitual use of nonsteroidal anti-inflammatory drugs is associated with a lower CRC risk (Wang and DuBois 2013). Moreover, circulating inflammatory biomarkers, such as C-reactive protein (CRP) (Aleksandrova et al. 2010), cytokines, chemokines, and cell adhesion molecules (McClellan et al. 2012; Song et al. 2013), and some genes related to pro-inflammation (Landi et al. 2003, 2007) tended to be associated with a higher CRC risk. Diet plays a crucial role in the etiology of CRC (World Research Cancer Fund and American Institute for Cancer Research 2007), although there is little evidence of the pro-inflammatory and anti-inflammatory effects of the overall diet on CRC risk (Shivappa et al. 2014a).

The dietary inflammation index (DII) is a literature review-based score that reflects the potential inflammatory effects of the diet. It was developed by Cavicchia et al. (2009) and updated by Shivappa et al. (2014b). In this new version, nearly 2,000 papers were reviewed and scored. Forty-five food parameters, including foods, nutrients, and other bioactive compounds were evaluated based on their inflammatory effect on some specific inflammatory markers, such as interleukin (IL)-1, IL-4, IL-6, IL-10, tumor necrosis factor (TNF)-α, and CRP. The DII has been validated demonstrating its effectiveness in predicting serum CRP levels in a large longitudinal epidemiological study (Shivappa et al. 2014c). Previously, we observed that women with a pro-inflammatory diet (higher DII scores) had a higher risk of developing CRC in a US-based cohort study (Shivappa et al. 2014a). Furthermore, higher DII scores have been linked to asthma (Wood et al. 2014), and using a modification of the previous DII version (Hebert et al. 2014), a positive association was observed between the DII and higher concentrations of glucose metabolism markers (van Woudenbergh et al. 2013).

The aim of the current study was to investigate the association between DII and CRC risk, and the potential interactions with some polymorphisms of inflammatory genes in a Spanish case–control study.

Subjects and methods

Study design and case ascertainment

The Bellvitge Colorectal Cancer Study is a hospital-based case–control study designed to investigate the relationships between risk factors of CRC and gene–environment interactions. The full rationale, methods, and design have been described previously (Landi et al. 2003). Briefly, primary CRC cases were recruited at the University Hospital of Bellvitge, Barcelona (Spain), between January 1996 and December 1998. A total of 523 histologically confirmed CRC cases were identified, of whom 424 participated in the study (81 % participation rate). Controls were randomly selected from admissions to the same hospital during this period. To minimize selection bias, the criterion of inclusion in the control group was a new disease (not previously diagnosed) for that patient. Twenty-two percent of controls were admitted for internal medicine, 19 % for acute surgery, 17 % for urology, 16 % for gastroenterology (hernia, peptic ulcer, and cholecystitis), 15 % for traumatology, and 11 % for circulatory or respiratory conditions. Controls were frequency-matched to cases by sex and age (±5 years). A total of 470 controls were approached, of whom 442 were deemed eligible and 401 agreed to participate in the study (85 % participation rate). All participants gave written consent, all procedures were in accordance with the Ethical standards of the Helsinki Declaration, and the Ethical Committee of the hospital approved the study protocol.

Dietary assessment

The participants' habitual diet in the year previous to diagnosis was recorded in a personal interview using a validated Spanish dietary history questionnaire (EPIC

Group of Spain 1997a, b). Energy, nutrient, and flavonoid intakes were estimated from the Spanish food composition tables used for the European Prospective Investigation into Cancer and Nutrition study (Slimani et al. 2007; Zamora-Ros et al. 2013a, b). Questionnaire-derived dietary information was used to calculate DII scores for all subjects, as described in detail elsewhere (Cavicchia et al. 2009; Shivappa et al. 2014b). Briefly, the dietary data for each study participant were first linked to the regionally representative global database that provided a robust estimate of a mean and standard deviation for each of the food parameters (i.e., foods, nutrients, and other food components such as flavonoids) considered (Shivappa et al. 2014b) to derive a *z*-score, by subtracting the "standard global mean" from the amount reported and dividing this value by the standard deviation. To minimize the effect of "right skewing" (a common occurrence with dietary data), this value was then converted to a centered percentile score which was then multiplied by the respective food parameter effect score (derived from a literature review and scoring of 1,941 articles) to obtain subject's food parameter-specific DII score. All of the food parameter-specific DII scores were then summed to create the overall DII score for every subject in the study (Supplementary Table 1). A positive score indicates a more pro-inflammatory diet, while a negative score reflects a diet that is more anti-inflammatory.

Gene and lifestyle assessment

Cases and controls were interviewed by trained personnel using structured questionnaires designed to collect information on sociodemographic characteristics, medical history, lifetime smoking habits, leisure- and work-related physical activity. Anthropometric data were measured, and a blood sample was taken.

The four selected SNPs of inflammatory genes [*IL-4*, *IL-6*, *IL-8*, and peroxisome proliferator-activated receptor-γ (*PPARG*)] were the genes significantly associated with CRC risk in our previous studies in the main effects or in the subgroup analyses (Landi et al. 2003, 2007). After DNA was extracted, genotyping was performed with the TaqMan technology using the protocol recommended by the supplier (Applied Biosystems, Foster City, CA, USA). The order of DNAs from cases and controls was randomized on PCR plates in order to ensure that a similar number of cases and controls were analyzed simultaneously in the same plate. Reactions were run in 96-well plates on a Tetrad DNA Engine PCR machine (MJ Research, Waltham, MA, USA) and read in a TaqMan 7900HT sequence detection system (Applied Biosystems, Foster City, CA, USA).

Statistical analysis

Characteristics of cases and controls were summarized as percentages of subjects for categorical variables and means and standard deviations for continuous variables. Distribution of DII score was assessed by the median (25th and 75th percentiles), because the data were skewed to the right.

The relationships between CRC risk and DII were assessed by estimating the odds ratios (OR) and 95 % confidence intervals (CIs) using an unconditional logistic regression, because the controls were frequency-matched to cases. DII score was included in the models as quartiles (categorically) based on the distributions among controls. To account for potential confounding and adjust for slight differences in the distribution of sex between cases and controls (Table 1), model 1 was adjusted for sex, age (years, continuous), and total energy intake (kcal/day, continuous). Model 2 was additionally adjusted for body mass index (kg/m^2, continuous), tobacco consumption (former, current, and never smoker), level of physical activity (no activity, low, and high), regular medications (aspirin, nonsteroidal anti-inflammatory drug, both, and none), and first-degree family history of CRC (yes, no). Tests for linear trend were performed by assigning the medians of each quartile as scores. DII score was also analyzed as a continuous variable (one unit of DII increment). The primary analysis was performed for all CRC combined; secondary analyses were carried out for colon and rectal cancers separately. The Wald test was used to evaluate the association and heterogeneity between cancer sites.

Diet–gene associations were tested by investigating the relationships between DII score (continuous) and CRC risk by SNPs of inflammatory genes using unconditional logistic regression and adjusting for the same variables as in model 2. Association between gene polymorphisms and DII score was assessed only in the control population using a linear regression model adjusted for covariate as model 2 before to test if genotype frequencies followed Hardy–Weinberg equilibrium. This association was also assessed in the complete sample. Partial Pearson correlation coefficients for DII and polymorphisms were derived from the linear models. Interactions between DII score and gene polymorphisms in relation to CRC risk were tested using the likelihood ratio test from logistic regression models with and without the interaction terms. Case-only analysis, though more powerful, was not considered because DII score was associated with some polymorphisms among controls. We used Bonferroni correction to account for multiple test and used a *P* value of 0.0125 (0.05–4) to indicate statistical significance. All statistical tests were two-tailed and were performed using the SPSS package program, version 17.0 (SPSS, Chicago, IL) and the genetic

Table 1 Characteristics of 424 colorectal cancer cases and 401 controls by quartiles of dietary inflammation index score in the Bellvitge Colorectal Cancer Study

	All	Q1	Q2	Q3	Q4
Cutoff		<−0.73	−0.73 to 1.06	1.07 to 3.05	>3.05
N					
Cases	424	112	81	114	117
Controls	401	101	101	98	101
Age (years)[a]					
Cases	66.2 (11.7)	63.8 (10.7)	65.1 (11.5)	67.5 (10.6)	68.0 (13.2)
Controls	65.1 (12.5)	64.1 (11.0)	63.8 (11.9)	64.2 (12.8)	68.2 (13.7)
Men (%)					
Cases	60.1	80.4	66.7	57.0	39.3
Controls	51.6	66.3	59.4	49.0	31.7
BMI (kg/m^2)[a]					
Cases	25.9 (4.2)	26.5 (3.9)	25.7 (3.8)	26.0 (4.4)	25.3 (4.6)
Controls	27.0 (4.8)	26.9 (4.9)	27.9 (4.3)	27.1 (5.4)	26.0 (4.6)
Current smokers (%)					
Cases	17.7	22.3	22.2	14.9	12.8
Controls	14.7	11.9	17.8	16.3	12.9
High physical activity (%)					
Cases	51.3	52.7	56.3	52.6	45.3
Controls	51.4	52.5	57.4	48.0	47.5
History of colorectal cancer[b] (%)					
Cases	15.3	17.9	13.6	13.2	16.2
Controls	5.0	8.9	3.0	5.1	3.0
Energy (kcal/day)[a]					
Cases	2,175 (793)	2,713 (860)	2,317 (743)	1,983 (618)	1,748 (563)
Controls	1,969 (678)	2,405 (725)	1,972 (594)	1,929 (630)	1,569 (469)
Alcohol (g/day)[a]					
Cases	12.0 (37.6)	31.8 (64.9)	7.6 (25.3)	15.0 (38.4)	2.1 (14.2)
Controls	9.5 (36.5)	14.4 (49.2)	12.7 (41.7)	8.4 (35.5)	4.9 (20.3)
Fruit and vegetables (g/1,000 kcal day)[a]					
Cases	230 (134)	302 (145)	240 (135)	228 (125)	157 (86)
Controls	274 (160)	364 (174)	299 (154)	245 (134)	190 (119)
Red and processed meat (g/1,000 kcal day)[a]					
Cases	38.3 (21.6)	38.5 (21.2)	42.0 (23.8)	36.5 (21.5)	37.1 (20.4)
Controls	39.5 (22.8)	38.0 (24.8)	42.1 (24.4)	38.9 (19.5)	38.9 (22.3)
Aspirin (%)					
Cases	16.3	18.8	17.3	15.8	13.7
Controls	18.7	13.9	18.8	15.3	26.7
NSAID (%)					
Cases	5.7	3.6	6.2	5.3	7.7
Controls	14.5	10.9	19.8	12.2	14.9

[a] Mean (SD)

[b] First-degree family history of colorectal cancer

epidemiology web tool SNPstats (http://www.snpstats.net) (Sole et al. 2006).

Results

A total of 424 CRC patients (265 and 159 with colon and rectal cancer, respectively) and 401 hospital-based control subjects were included in the current study. The medians (25th and 75th percentiles) of DII score were 1.44 (−0.88 and 3.18) and 1.06 (−0.73 and 3.05) for cases and controls, respectively. Age at recruitment and percentage of women were higher in the fourth quartile compared with the first (Table 1). In addition, subjects in the highest quartile tended to smoke less, particularly in cases, and to be less physically active. Furthermore, participants in the top quartile reported the lowest intake of total energy, alcohol, and fruit and vegetables (per 1,000 kcal).

In both multivariable logistic models, significant direct associations were observed between DII score and CRC risk ($OR_{Q4\ vs.\ Q1}$ 1.65, 95 % CI 1.05–2.60, and *P* trend 0.011) and colon cancer risk ($OR_{Q4\ vs.\ Q1}$ 2.24, 95 % CI 1.33–3.77, and *P* trend 0.002), but not with rectal cancer risk ($OR_{Q4\ vs.\ Q1}$ 1.12, 95 % CI 0.61–2.06, and *P* trend 0.37) (Table 2). Similar results were found when DII score was evaluated as a continuous variable. However, no significant heterogeneity between colon and rectal cancer risk was detected (*P* heterogeneity = 0.19).

In the diet–gene analysis, a significant correlation between DII score and *IL-4* rs2243250 polymorphism (partial $r = -0.34$, $P = 0.009$) was found among the control group. No significant correlation were observed with *IL-6* (partial $r = 0.20$, $P = 0.06$), *IL-8* (partial $r = 0.15$, $P = 0.18$), and *PPARG* (partial $r = -0.06$, $P = 0.63$) polymorphisms. Similar associations were observed in the complete dataset. A significant interaction was observed between DII score and *IL-4* genotype in relation to CRC risk. Multivariable logistic models evaluating the association between DII score and CRC risk stratified by SNP of inflammatory genes are presented in Table 3. The DII score was not associated with CRC for individuals homozygous CC for rs2243250 in *IL-4*, but the DII score was associated with a significant increased risk of carriers of the T allele (dominant model) (OR 1.34, 95 % CI 1.14–1.57). No significant interaction was observed for *IL-6*, *IL-8*, or *PPARG*.

Discussion

In the present case–control study, a statistically significant direct association was observed between CRC risk and DII score in a dose-dependent manner. CRC risk was increased by 51 and 65 % when participants in the third and the fourth DII quartile, respectively, were compared with those in the first quartile. Similar results were previously observed in the Iowa Women's Health Study, although the CRC risk, in this cohort, was only increased by 20 % (Shivappa et al. 2014a). Despite the limited evidence on the relationship between overall inflammatory effects of diet and CRC risk, other epidemiological studies have reported comparable associations between CRC risk and anti-inflammatory foods (e.g., fruits and vegetables) (World Research Cancer Fund and American Institute for Cancer Research 2007), nutrients (e.g., fiber, selenium, and folate) (van Duijnhoven et al. 2009), and other bioactive compounds (e.g., flavonoids) (Zamora-Ros et al. 2013b). In addition, higher circulating CRP and cytokine levels (inflammatory markers) have been associated with increased CRC risk in case–control studies, but in cohort studies, these associations have been less conclusive

Table 2 Association between dietary inflammation index (DII) score and risk of colorectal cancer in the Bellvitge Colorectal Cancer Study

	Cutoff	Colorectal cancer			Colon cancer			Rectal cancer		
		Cases	OR (95 % CI)[a]	OR (95 % CI)[b]	Cases	OR (95 % CI)[a]	OR (95 % CI)[b]	Cases	OR (95 % CI)[a]	OR (95 % CI)[b]
Q1	<−0.73	112	1	1	66	1	1	46	1	1
Q2	−0.73 to 1.06	81	0.89 (0.59–1.35)	0.96 (0.63–1.48)	55	1.09 (0.68–1.76)	1.25 (0.76–2.06)	26	0.65 (0.37–1.16)	0.71 (0.39–1.29)
Q3	1.07–3.05	114	1.44 (0.95–2.16)	1.51 (0.99–2.31)	66	1.47 (0.91–2.37)	1.58 (0.96–2.59)	48	1.36 (0.81–2.29)	1.48 (0.85–2.55)
Q4	>3.05	117	1.66 (1.08–2.56)	1.65 (1.05–2.60)	78	2.02 (1.23–3.33)	2.24 (1.33–3.77)	39	1.20 (0.67–2.14)	1.12 (0.61–2.06)
P trend			0.008	0.011		0.004	0.002		0.25	0.37
Continuous (1 DII unit)		424	1.08 (1.02–1.15)	1.08 (1.01–1.15)	265	1.10 (1.03–1.19)	1.12 (1.04–1.21)	159	1.04 (0.96–1.13)	1.03 (0.95–1.12)

[a] Model 1 was adjusted for sex, age, and total energy intake

[b] Model 2 was additionally adjusted for body mass index, first-degree family history of colorectal cancer, physical activity, tobacco consumption, and medication use (aspirin and nonsteroidal anti-inflammatory drug)

Table 3 Associations between dietary inflammatory index score (continuous) and colorectal cancer risk by polymorphisms in inflammatory genes in the Bellvitge Colorectal Cancer Study

Gene	Rs number	Trivial name	Cases	Controls	OR (95 % CI)[a]	*P* for interaction[b]
IL4[c]	rs2243250	−588 C>T				0.004
CC			209	207	1.04 (0.95–1.13)	
CT+TT			65	59	1.34 (1.14–1.57)	
IL6[c]	rs1800795	174 G>C				0.26
GG			131	143	1.12 (1.01–1.24)	
GC+CC			222	163	1.04 (0.96–1.13)	
IL8[c]	rs4073	−251 T>A				0.85
TT			114	81	1.11 (0.98–1.25)	
TA+AA			230	222	1.09 (1.01–1.19)	
PPARG[c]	rs1801282	34 C>G				0.49
CC			305	238	1.09 (1.01–1.17)	
CG+GG			46	65	1.03 (0.88–1.20)	

IL interleukin, *PPARG* peroxisome proliferator-activated receptor-γ

[a] Increase in CRC risk for each unit of DII score. Adjusted for sex, age, total energy intake, body mass index, first-degree family history of colorectal cancer, physical activity, tobacco consumption, and medication use (aspirin and nonsteroidal anti-inflammatory drug)

[b] Differences in risk associated to dietary inflammatory index score by genotype

[c] Not available data for some individuals produce OR estimates different from the complete dataset shown in Table 2 (OR 1.08, 95 % CI 1.01–1.15). Missing values for *IL-4*: 150 cases and 135 controls, for *IL-6*: 71 cases and 95 controls, for *IL-8*: 80 cases and 98 controls, and for *PPARG*: 72 cases and 98 controls

(Aleksandrova et al. 2010; Song et al. 2013; Wu et al. 2013).

Our results suggest that the association of DII score with colon cancer risk could be stronger than with rectal cancer risk, but the interaction was not statistically significant. In other epidemiological studies, similar associations were reported for colon and rectal cancer risks with DII score (Shivappa et al. 2014a), and intakes of fruits and vegetables, fiber, and flavonoids (World Research Cancer Fund and American Institute for Cancer Research 2007; Zamora-Ros et al. 2013b; Murphy et al. 2012). However, for circulating CRP levels, significant associations were observed only for colon cancer risk (Aleksandrova et al. 2010; Wu et al. 2013). Although colon and rectal cancers may have different etiologies (Wei et al. 2004), our study did not show large differences between colon and rectal cancer in the effect of inflammation. Lack of statistically significant findings for rectal cancer may be a result of smaller sample size for rectal cancer.

In previous reports from our case–control study, SNPs in the *IL-4*, *IL-6*, *IL-8*, and *PPARG* genes related to inflammation pathways were associated with CRC risk (8; 9). For *IL-4*, the main effect was not statistically significant in the complete dataset (OR 1.23, 95 % CI 0.81–1.86), but was significant in the colon cancer subgroup (Landi et al. 2007). Our results have shown that individuals with a T allele in the rs2243250 SNP, located in the promoter region of *IL-4*, tended to have a lower DII. However, when these individuals had a high DII, their risk of CRC was significantly increased. This effect was not observed in individuals with the more frequent C allele. It is interesting that this SNP has been associated with diverse diseases related to inflammation, including cancer. In some studies, the T allele of this SNP was related to an increased disease risk, such as liver diseases (Zheng et al. 2013), renal cell cancer (Zhenzhen et al. 2013), and asthma (Liu et al. 2012). On the other hand, the T allele of this SNP was associated with a decrease risk in oral cancer (Zhenzhen et al. 2013) and myocardial infarct in young people (Paffen et al. 2008). We hypothesize that, in individuals with the T allele, the activity of the cytokine IL-4 may be downregulated. In diets with a low DII, protective effects of the IL-4 pathway might be compensated by other anti-carcinogenic and anti-inflammatory pathways. However, in diets with a high DII, these alternative pathways may be not enough, and therefore, the CRC risk was higher than in subjects with the C allele of this SNP. Further studies evaluating the association of inflammatory markers and CRC risk by SNP in inflammatory genes are needed to confirm our findings.

The first step of colorectal carcinogenesis occurs in an inflammatory environment wherein infiltrating lymphocytes and macrophages raise the level of reactive oxygen and nitrogen spices and stimulate release of pro-inflammatory grown factors, cytokines, and chemokines (Coussens and Werb 2002). Microbiota (Candela et al. 2014) and a healthy diet (Wang et al. 2012) play an important role in

keeping intestinal mucosa in a state of low-grade inflammation. However, when this is chronically activated, the inflammatory/oxidative environment becomes a relentless cycle that results in genetic and pathological damage. All food components included in the DII score have been inversely or positively associated with inflammation (Shivappa et al. 2014b), and therefore, their inclusion in a dietary score is crucial to properly evaluate the complex association between diet-related inflammation and CRC risk.

We are aware that in any case–control study, there are potential limitations such as reverse-causality and that the use of hospital controls is not ideal, though there is some evidence that hospital controls may be superior to population controls (especially, when the base population is difficult to delineate) (Infante-Rivard 2003). Firstly, we tried to minimize measurement error by using validated questionnaires administered by trained interviewers (EPIC Group of Spain 1997a, b). Despite that, intakes of some dietary components, which were included in the previously published DII (Shivappa et al. 2014b), such as caffeine, eugenol, ginger, saffron, selenium, pepper, thyme,oregano, and rosemary, could not be calculated from our dietary history. However, the variation in intakes of those specific dietary components was expected to be low in a mostly non-vegetarian Spanish population. Secondly, although extensive information about potential confounders was available, residual confounding might have remained because potential confounders could have been measured with error. Thirdly, the use of hospital controls may have resulted in a selected control group with potentially different prevalence of inflammatory alleles than the reference population. However, it has previously shown that hospital controls have minimal effect on the allele frequencies (Garte et al. 2001).

In conclusion, we found that high-DII diets are associated with increased risk of CRC in a hospital-based case–control study in Spain, which was more pronounced for colon cancer than for rectal cancer. The positive association differed according to the genotype of rs2243250 in the promoter region of the inflammatory gene *IL-4*. Future studies are needed to evaluate the potential use of DII as a global measure of inflammatory potential of diet in relation to CRC risk in prospective studies and its relation to genetic susceptibility.

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