The *D1822V APC* polymorphism interacts with fat, calcium, and fiber intakes in modulating the risk of colorectal cancer in Portuguese persons[1–3]

Catarina S Guerreiro, Marília L Cravo, Miguel Brito, Pedro M Vidal, Paulo O Fidalgo, and Carlos N Leitão

ABSTRACT

Background: Both genetic and environmental factors affect the risk of colorectal cancer (CRC).

Objective: We aimed to examine the interaction between the *D1822V* polymorphism of the *APC* gene and dietary intake in persons with CRC.

Design: Persons with CRC ($n = 196$) and 200 healthy volunteers, matched for age and sex in a case-control study, were evaluated with respect to nutritional status and lifestyle factors and for the *D1822V* polymorphism.

Results: No significant differences were observed in energy and macronutrient intakes. Cases had significantly ($P < 0.05$) lower intakes of carotenes, vitamins C and E, folate, and calcium than did controls. Fiber intake was significantly ($P = 0.004$) lower in cases than in controls, whereas alcohol consumption was associated with a 2-fold risk of CRC. In addition, cases were significantly ($P = 0.001$) more likely than were controls to be sedentary. The homozygous variant for the *APC* gene (*VV*) was found in 4.6% of cases and in 3.5% of controls. Examination of the potential interactions between diet and genotype found that a high cholesterol intake was associated with a greater risk of colorectal cancer only in noncarriers (*DD*) of the *D1822V APC* allele (odds ratio: 1.66; 95% CI: 1.00, 2.76). In contrast, high fiber and calcium intakes were more markedly associated with a lower risk of CRC in patients carrying the polymorphic allele (*DV/VV*) (odds ratio: 0.50; 95% CI: 0.27, 0.94 for fiber; odds ratio: 0.51; 95% CI: 0.28, 0.93 for calcium) than in those without that allele.

Conclusion: These results suggest a significant interaction between the *D1822V* polymorphism and the dietary intakes of cholesterol, calcium, and fiber for CRC risk. *Am J Clin Nutr* 2007;85: 1592–7.

KEY WORDS Colorectal cancer, diet, genetic polymorphism, lifestyle factors

INTRODUCTION

Colorectal cancer (CRC) is a major cause of cancer mortality in industrialized countries that have adopted a Western type of diet (1). Both environmental and genetic factors have been implicated in the occurrence of CRC (2). Of environmental factors, obesity, a lack of physical activity, high energy intake, and a diet rich in red meat and poor in protective factors such as fruit and vegetables have consistently been associated with a greater risk of CRC (3, 4).

However, different studies show conflicting results with respect to the factors that protect from and those that promote CRC: although most case-control studies support the abovementioned associations (5), the results obtained in prospective, interventional studies are less clear-cut. The reasons for these discrepancies may relate to recall bias, which occurs in case-control studies because, in a disease such as cancer, dietary intake in the more distant past may be more relevant but more difficult to recall (6). Moreover, in an assessment of cancer risk, there are complex dietary interactions that occur not only between nutrients but also with other variables, such as smoking and drinking habits, exercise or body composition, or both, that confound the analysis of a single nutrient. In their review, Marques-Vidal et al (5) observed that the isolated consumption of either vegetables or fruit conferred no protection in most of the studies analyzed, whereas the combined consumption of both of these foodstuffs was protective.

Moreover, these dietary relations are further complicated by interactions with genetic factors. This genetic heterogeneity of the studied population may explain why different persons respond differently to similar nutritional stimuli. A difference in susceptibility is usually due to the presence of single-nucleotide polymorphisms (SNPs), which consist of mutations in single DNA bases. These SNPs do not always affect protein function but may account for variations such as greater protection from or susceptibility to a certain disease, especially in conjunction with environmental stimuli such as dietary intake (7).

With respect to CRC, a polymorphism in the *APC* gene (*Adenomatous polyposis coli*) has been studied that, when correlated with nutritional factors, may influence the risk of CRC. This polymorphism is characterized by a substitution of an adenine

[1] From the Escola Superior de Tecnologia da Saúde de Lisboa, Lisbon, Portugal (CSG and MB); the Serviço de Gastrenterologia do Instituto Português de Oncologia de Lisboa Francisco Gentil Entidade Publica Empresarial, Lisbon, Portugal (MC, PF, and CNL); the Unidade de Nutrição e Metabolismo Instituto de Medicina Molecular da Universidade de Lisboa, Lisbon, Portugal (CSG and PMV).

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[3] Reprints not available. Address correspondence to C Sousa Guerreiro, Área Cientifica de Dietética, Escola Superior de Tecnologia da Saúde de Lisboa, Av. D. João II, Lote 4.69.01 1990-096 Lisbon, Portugal. E-mail: catarina.guerreiro@estesl.ipl.pt.

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base for a thymidine base in codon 1822, which results in a substitution of an aspartate base for a valine base (GAC→GTC) (8). The interaction between dietary habits and this polymorphism in CRC development has been examined in 2 studies, but the examination for nutritional correlates was restricted to fat intake (9, 10). The aim of the present study was to assess the interaction between the *D1822V* polymorphism in the *APC* gene and dietary intake in a group of Portuguese CRC patients.

SUBJECTS AND METHODS

Subjects

This was a case-control study, performed at the Instituto Português de Oncologia de Lisboa Francisco Gentil EPE (IPOLFG). The group with cancer (ie, cases) was composed of 196 subjects (104 M, 92 F; $\bar{x} \pm$ SD age: 64.2 ± 11.3 y) with a histologic diagnosis of CRC, and the control group included 200 healthy blood donors and health care workers recruited from IPOLFG, with a similar sex and age distribution (106 M, 94 F; age 62.2 ± 12.1 y) and no history of cancer at any site. Of the cases, 169 of 196 (86.5%) had a recent diagnosis of CRC, and the remaining 27 (13.5%) were being treated for a disease relapse. With respect to previous therapies, 119 of 196 (60.7%) had not received any form of treatment, 28 of 196 (14.3%) had already undergone surgery, 13 of 196 (6.6%) had had pelvic radiotherapy, 11 of 196 (5.6%) had received ≥1 cycles of chemotherapy, and the 25 of 196 (12.8%) had undergone combined forms of treatment. TNM staging was as follows: stage I, 24 of 178 (13.5%); stage II, 64 of 178 (35.9%); stage III, 53 of 178 (29.8%); and stage IV, 37 of 178 (20.8%).

All subjects gave written informed consent. The study was approved by the Scientific Ethics Committee of the IPOLFG.

Data collection

Dietary assessment was performed by using a food-frequency questionnaire (11), which was validated for the Portuguese population. Participants were asked to recall their habits in the year before CRC diagnosis (cases) or in the year before interview (controls). The type and quantity of food intake were then analyzed in a modified database FOOD PROCESSOR software (version 7; Esha Research, Inc, Salem, OR) including some Portuguese food items, which allows the quantification of different macronutrients and micronutrients. Physical activity in the year before cancer diagnosis (cases) or in the year before interview (controls) was assessed by using the questionnaire of Arroll et al (12), which groups subjects in 3 categories: low (0), intermediate (1), and high (2) activity. Smoking habits were recorded as the number of cigarettes smoked per day. For calculation of the body mass index (BMI; in kg/m^2), we used a digital scale (SECA, Hanover, MD) that was accurate to ±0.1 kg and the height that had been recorded in the participants' government identity cards.

Genotype data

Whole blood samples were collected and stored in 3-mm Cards Kit papers. DNA extraction was done by using a Generation Capture Card Kit–DNA Purification; DNA Elution (all: Gentra Systems Inc, Minneapolis, MN). The GAC→GTC polymorphism at codon 1822 of the *APC* gene was genotyped by using an Assays-by-Design TaqMan allelic discrimination assay (Applied Biosystems, Foster City, CA). Polymerase chain reaction (PCR) was carried out with mixes consisting of 4 μL genomic DNA, 10 μL Taqman Universal PCR Master Mix (Applied Biosystems,), 1 μL of 20× assay mix, and ddH_2O up to 20 μL of final volume. The following amplification protocol was used: denaturation at 95 °C for 10 min, 40 cycles of denaturation at 92 °C for 15 s, and annealing and extension at 60 °C for 1 min.

All of the reactions were performed in the iCycler iQ Multicolor Real Time PCR Detection System (Bio-Rad, Hercules, CA). After PCR, the genotype of each sample was attributed automatically by measuring the allele-specific fluorescence on the equipment software. Subjects were classified as homozygous for the variant if they carried both valine and valine (*VV*) alleles, heterozygous if they had the aspartate and valine (*DV*) genotype, and homozygous wild-type if they had the aspartate and aspartate (*DD*) genotype.

Statistical analysis

Statistical analysis was performed by using SPSS for WINDOWS software (version 12.0; SPSS Inc, Chicago, IL). Data were expressed as means ± SDs, as the number (and percentage) of subjects, and as odds ratio (OR) and 95% CIs. Bivariate analyses were conducted by using Student's *t* test or the Mann-Whitney test for continuous variables and the chi-square test for categorical variables. Multiple logistic regression was used to estimate associations by using ORs and 95% CIs. Briefly, dietary habits were categorized as high or low for intake above or below the median values, respectively, and 4 independent groups were created according to *D1822V APC* and diet. Risk was assessed by examining the combined effects of the *D1822V* genotype and diet, using as the referent category (ie, standard risk) those with low dietary intake and no variant for *APC* gene. The remaining 3 groups were no variant and high intake, with variant (*DV* or *VV*) and low intake, and with variant and high intake. Those groups were then included in the logistic regression to assess their relations with risk of CRC; further adjustment on other variables was performed whenever necessary. Statistical significance was established for $P < 0.05$.

RESULTS

Lifestyle habits, family history, weight, and BMI for both study groups are shown in **Table 1**. With respect to physical activity, cases had lower levels of intense activity before cancer diagnosis than after, but exercise per se did not influence the risk of developing CRC (OR: 0.78; 95% CI: 0.53, 1.1). Moreover, no differences in smoking habit were found between groups. With respect to alcohol intake, we observed that 35.8% of patients and 38.5% controls did not consume any alcohol. Of the subjects who drank alcohol, cases had a significantly higher alcohol than did controls (38.8 g/d compared with 31.0 g/d; OR: 1.97; 95% CI: 1.19, 3.26). Familial history of CRC in first- or second-degree relatives was also greater in CRC subjects than in controls (OR: 3.4; 95% CI: 1.85, 6.29). With respect to nutritional assessment, we observed that both weight and BMI were significantly lower in cases than in controls (Table 1).

Daily energy and macronutrient intakes are shown in **Table 2**. No significant differences in macronutrient consumption were observed between cases and controls. However, we observed a significantly lower intake of fiber in cases than in controls: those with a daily intake higher than the median (25.6 g) had an OR of

TABLE 1
Characteristics of study sample[1]

	Cases (n = 196)	Controls (n = 200)	P
Physical activity[2]			
Low [n (%)]	106 (54.1)	96 (48.0)	—
Intermediate [n (%)]	78 (39.8)	66 (33.0)	—
High [n (%)]	12 (6.1)	38 (19.0)	<0.001[3]
Smoker [n (%)]	24 (12.2)	26 (13.0)	NS
Cigarettes (no./d)[3]	7.0 ± 12.4[4]	7.9 ± 13.6	NS
Drinker [n (%)]	127 (64.8)	123 (61.5)	NS
Ethanol (g/d)	25.2 ± 39.8	19.2 ± 43.4	NS
Familial CRC history [n (%)][5]	45 (23)	16 (8)	0.001
Height (m)	1.63 ± 0.09	1.64 ± 0.09	NS
Weight (kg)	68.5 ± 13.9	72.3 ± 12.1	0.004
BMI (kg/m^2)	25.6 ± 4.2	27.0 ± 3.9	0.01

[1] CRC, colorectal cancer. For Physical activity, drinkers, smokers, and familial CRC history, a chi-square test was used for statistical analysis; differences in ethanol intake were analyzed by using the Mann-Whitney U test; and differences in height, weight, and BMI between groups were analyzed by using Student's t test.
[2] Classified according to the questionnaire of Arroll et al (12).
[3] The prevalence of high physical activity was significantly greater in controls than in cases.
[4] $\bar{x}$ ± SD (all such values).
[5] CRC in first- or second-degree relative.

0.58 (95% CI: 0.4, 0.8) for CRC development compared with those who consumed less than the median.

Micronutrient intake in both study groups—including supplemental intake, which occurred in 11 cases and no controls—is shown in **Table 3**. We observed that cases had a significantly lower intake of carotenes, vitamin C, vitamin E, folate, and calcium than did controls. For these micronutrients, ORs were calculated considering intake above or below the median.

No significant differences in genotype distribution were observed, and both groups were found to be in Hardy-Weinberg equilibrium. Homozygotes for the variant made up 4.6% of cases and 3.5% of controls, whereas heterozygotes made up 30.1% and 33% of cases and controls, respectively. In the cases only, we observed no correlation between genotype and sex, tumor location, stage of disease, or familial history of CRC. The interaction between intake of several macronutrients and micronutrients and *APC* genotype is shown in **Table 4**. To increase statistical power, risk was assessed by combining the heterozygotes and variant homozygotes against the reference category of homozygosity for the wild-type allele. Significant associations were observed between genotype and cholesterol (P = 0.05), fiber (P = 0.03), and calcium (P = 0.03) intakes. A high intake of cholesterol was associated with a significantly (P = 0.05) greater risk of developing CRC only in wild-type (*DD*) carriers, whereas high intakes of fiber and calcium decreased the risk of developing CRC by nearly 50% in cases with the polymorphic alleles (*DV* and *VV*). Separate consideration of the 3 genotypes resulted in somewhat different P values for interaction, but the fundamental interaction between *APC* genotype and fat, calcium, and fiber intakes still held true.

TABLE 2
Daily intake of macronutrients[1]

	Cases (n = 196)	Controls (n = 200)	P[2]
Energy (kcal)	3113 ± 1178	2938 ± 1015	NS
Proteins (g)	133.5 ± 57.6	130.3 ± 50.4	NS
Carbohydrates (g)	285.6 ± 86.1	290.0 ± 95	NS
Total fat (g)	134.0 ± 77.7	130.0 ± 95	NS
Polyunsaturated fat (g)	21.2 ± 12.1	19.9 ± 9.5	NS
Monounsaturated fat (g)	54.5 ± 28.5	54.5 ± 25.0	NS
Saturated fat (g)	47.6 ± 34.4	45.3 ± 30.9	NS
Total cholesterol (mg)	507 ± 304.7	460 ± 250.3	NS
Fiber (g)	26.0 ± 9.3	28.9 ± 10.7	0.004

[1] All values are $\bar{x}$ ± SD.
[2] The Mann-Whitney U test was used to analyze differences between groups.

DISCUSSION

Numerous studies have examined the relation between diet, lifestyle habits, and genetic traits and the prevalence of CRC, and the results obtained have varied widely. The present study was aimed at correlating dietary intake with the *D1822V* polymorphism in the *APC* gene in a group of Portuguese subjects. Although this missense mutation does not theoretically affect protein function, it may result in slight conformational alterations that may interact with environmental factors, thereby influencing the risk of developing CRC.

We observed that the proportion of subjects with intense physical activity was lower among the cases than among the controls. However, in contrast to previous studies (13, 14), we did not observe that smokers were at higher risk of CRC. The latter finding may be related to the fact that the present study had a smaller sample than did previous studies. Analysis of alcohol intake showed that daily consumption >28.9 g alcohol/d nearly doubled the risk of CRC. In this respect, the literature is somewhat confusing, because some studies have reported a promoting effect of alcohol intake (15), although others have not (16), and still others have found a promoting effect only for rectal cancer (17).

Assessment of nutritional status found that cases had a BMI lower than that of controls. As stated before, this evaluation was performed after disease diagnosis, and 40% of patients had already undergone some form of medical or surgical treatment (or both). It is plausible, therefore, that complaints related to CRC or its treatment (or both) may have had a negative effect on the nutritional status of some patients. Kune et al (18) reported findings similar to our own with respect to BMI: in a large case-control study involving 715 cases matched to 727 controls, they did not find that excess of body weight was a risk factor for CRC. This is in contrast to several prospective studies that found increased BMI to be a risk factor for CRC (19, 20); in those studies, excess body weight was detected long before diagnosis or treatment (or both) took place.

No differences in macronutrient intake were present, but significant differences were observed with respect to the intake of micronutrients. We found that intakes of carotenes, vitamin C, vitamin E, folate, calcium, and fiber were significantly lower in cases than in controls. OR calculations showed a significant protective effect for fiber, calcium, and carotenes. Most studies

TABLE 3
Daily intake of micronutrients[1]

	Cases (n = 196)	Controls (n = 200)	P[2]	OR (95% CI)[3]
Vitamin A (μg)	944.8 ± 890.0	925.4 ± 644	NS	ND
Carotenes (μg)	985.5 ± 59.9	1264.5 ± 1128.3	0.02	0.67 (0.44, 0.99)[4]
Vitamin C (mg)	144.9 ± 60	168.4 ± 74.8	0.001	0.56 (0.38, 0.84)[4]
Vitamin D (μg)	7.01 ± 4.7	7.2 ± 4.7	NS	ND
Vitamin E (mg)	10.8 ± 4.4	11.9 ± 5.1	0.03	0.75 (0.5, 1.1)
Vitamin K (μg)	4353.8 ± 1363.7	4408.6 ± 1335.7	NS	ND
Folate (μg)	401.6 ± 161.9	433.4 ± 162.0	0.02	0.72 (0.49, 1.1)
Vitamin B-6 (mg)	2.83 ± 1.06	2.85 ± 0.98	NS	ND
Vitamin B-12 (μg)	14.54 ± 9.05	14.3 ± 8.21	NS	ND
Sodium (mg)	3961.9 ± 2847.3	3662.43 ± 2282.3	NS	ND
Potassium (mg)	4353.8 ± 1363.7	4408.6 ± 1335.7	NS	ND
Magnesium (mg)	416.6 ± 134.9	428.5 ± 131.1	NS	ND
Selenium (mg)	144.5 ± 56.3	143.5 ± 53.6	NS	ND
Iron (mg)	22.2 ± 9.8	21.8 ± 8.7	NS	ND
Calcium (mg)	1029.3 ± 410.7	1181.3 ± 626.4	0.01	0.59 (0.49, 0.88)[4]

[1] All values are $\bar{x}$ ± SD. OR, odds ratio; ND, not determined.
[2] The Mann-Whitney U test was used to analyze differences between groups.
[3] ORs were determined by using the values above or below median nutrient intake. Reference category—ie, standard risk for colorectal cancer—was considered a combination of the wild-type genotype and low intakes of several micronutrients. Cutoffs were 873.4 μg for carotenes, 147.5 mg for vitamin C, 10.3 mg for vitamin E, 406.7 μg for folate, and 1029 mg for calcium.
[4] $P < 0.05$.

are consistent with our observations on calcium intake, finding a protective effect of calcium intake against adenoma recurrence (21) and against the development of CRC (22). In a meta-analysis involving 10 studies aimed at examining the effect of calcium intake on CRC risk, Eunyoung et al (23) observed that an intake of >1100 mg Ca/d reduces the risk of developing CRC by 25% (OR: 0.74; 95% CI: 0.62, 0.88). In the present study, we observed a 40% reduction in the risk of developing CRC for those consuming >1029 mg Ca/d.

The protective effect of antioxidants against colorectal carcinogenesis has been shown by some (24, 25) but not all (26) studies. The same applies to the protective effect of fiber that was observed in this study: our results are consistent with those of most case-control studies (27, 28), but they diverge from the results presented in prospective studies (29–31). These discrepancies may be related to the facts that the source of these nutrients are fruit and vegetables and that analysis of the isolated effect of a single nutrient might be misleading. This hypothesis is further supported by a recent review (5) in which the authors observed that consumption of both fruit and vegetables exerts a protective effect stronger than that of separate consumption of each of these foods.

Regarding the polymorphism of *APC* gene, the percentage of heterozygotes, wild types, and homozygous variants was similar between the cancer and control groups, which is also similar to 2 previously published studies (9, 10). This finding allows us to conclude that the polymorphism per se does not modify the risk of developing CRC. Some interesting results were observed with regard to the interaction between nutritional and genetic factors. To our knowledge, only 2 previous studies examined the interaction between this polymorphism, nutrition, and the risk of CRC (9, 10). Slattery et al (9) observed that persons who were homozygous for this variant were at lower risk if they consumed a low-fat diet (OR: 0.2; 95% CI: 0.1, 0.5). In another study, Menendez et al (10) did not observe any interaction between nutrients and the polymorphic allele. In the present study, we observed that a high intake of cholesterol resulted in a higher risk of CRC development only in subjects who were homozygous for the wild-type allele (*DD*). This promotor effect was not observed in carriers of the polymorphic allele. Moreover, we observed that fiber and calcium intakes were mainly protective [OR: 0.50 (0.27, 0.94); OR: 0.51 (0.28, 0.93), respectively] for both heterozygous and homozygous carriers of the mutated allele. Neither of the 2 previous studies found any significant interaction with any nutrient besides fat.

At this moment, we can only speculate on possible mechanisms through which fat, calcium, or fiber intake can interact with *APC* polymorphism to modify the risk of CRC development. APC is a multifunctional protein, although its main tumor suppressor function appears to reside in the capacity to down-regulate β-catenin in the WNT signaling pathway. In the presence of *APC*-truncating mutations, β-catenin cannot be phosphorylated; it accumulates in the cytoplasm, from which it translocates to the nucleus, which leads to the activation of *WNT* target genes (32). Thus far, only 2 missence variants of the *APC* gene—ie, I1307K and E1317Q variants—have been associated with increased risk of CRC, although the association with the latter is still controversial (33). Although missense variants in the *APC* gene are not potentially pathogenic, so far nothing has been reported for the *D1822V* variant. Thus, we cannot exclude the possibility that this amino acid alteration located between the 4th and the 5th β-catenin down-regulating domain may induce some conformational alterations in the APC protein that may lead to a slightly less efficient β-catenin down-regulation. One can hypothesize that the presence of this variant is not in itself sufficient for tumor initiation. However, in the presence of dietary factors

TABLE 4
Odds ratios (ORs) for colorectal cancer according to *D1822T* APC and diet[1]

	D1822T APC				
	Cases/controls		OR (95% CI)[2]		
Intakes	Without variant (*DD*)	With variant (*DV/VV*)	Without variant (*DD*)	With variant (*DV/VV*)	*P* for interaction
	n				
Energy					
High	54/62	27/37	1.29 (0.78, 2.12)	0.90 (0.48, 1.68)	0.21
Low	74/65	41/36	1	1.20 (0.67, 2.16)	
Protein					
High	73/59	26/37	1.57 (0.95, 2.60)	0.91 (0.49, 1.71)	0.24
Low	55/68	42/36	1	1.42 (0.79, 2.56)	
Carbohydrates					
High	69/67	26/35	1.15 (0.70, 1.90)	0.91 (0.48, 1.71)	0.50
Low	59/60	42/38	1	1.06 (0.59, 1.89)	
Total fat					
High	72/61	23/42	1.37 (0.83, 2.27)	0.69 (0.37, 1.3)	0.12
Low	56/66	45/31	1	1.59 (0.89, 2.88)	
Saturated fat					
High	72/62	23/41	1.28 (0.78, 2.12)	0.67 (0.36, 1.26)	0.17
Low	56/65	45/32	1	1.51 (0.84, 2.73)	
Polyunsaturated fat					
High	76/63	21/38	1.48 (0.89, 2.45)	0.74 (0.38, 1.42)	0.14
Low	52/64	47/35	1	1.55 (0.87, 2.78)	
Monounsaturated fat					
High	72/61	27/38	1.40 (0.85, 2.32)	0.89 (0.48, 1.66)	0.10
Low	56/66	41/35	1	1.31 (0.73, 2.35)	
Total cholesterol					
High	77/60	26/35	1.66 (1.0, 2.76)	1.00 (0.53, 1.89)	0.05
Low	51/67	42/38	1	1.40 (0.78, 2.50)	
Fiber					
High	61/73	23/41	0.7 (0.43, 1.16)	0.50 (0.27, 0.94)	0.03
Low	67/54	45/32	1	1.08 (0.60, 1.95)	
Alcohol					
High	65/62	35/36	1.14 (0.69, 1.88)	1.06 (0.58, 1.91)	0.96
Low	63/65	33/37	1	0.90 (0.50, 1.65)	
Folate					
High	61/66	27/44	0.90 (0.54, 1.48)	0.60 (0.33, 1.09)	0.13
Low	67/61	41/29	1	1.28 (0.70, 2.32)	
Vitamin B-6					
High	66/64	26/41	0.99 (0.59, 1.62)	0.66 (0.35, 1.21)	0.16
Low	62/63	42/32	1	1.23 (0.67, 2.24)	
Vitamin B-12					
High	72/62	26/38	1.35 (0.82, 2.22)	0.85 (0.45, 1.58)	0.09
Low	56/65	42/35	1	1.31 (0.73, 2.35)	
Calcium					
High	59/69	26/44	0.74 (0.45, 1.21)	0.51 (0.28, 0.93)	0.03
Low	69/58	42/29	1	1.21 (0.66, 2.20)	

[1] $n = 128$ cases without variant; $n = 127$ controls without variant, $n = 68$ cases with variant, $n = 73$ controls with variant.

[2] ORs were estimated by examining the combined effect of *D1822V* genotype and dietary intake by using the values above or below the median nutrient intake. The cutoffs were 2827 for kcal, 121.1 g for protein, 270 g for carbohydrates, 110.9 g for total fat, 32.7 g for saturated fat, 48.3 g for monounsaturated fat, 17.6 g for polyunsaturated fat, 404 mg for cholesterol, 25.6 g for fiber, 3.4 g/d for alcohol, 12.5 μg for vitamin B-12, 406.7 μg for folate, and 12.5 mg for vitamin B-12. Reference category—ie, standard risk for colorectal cancer—was considered a combination of the wild-type genotype and low intakes of several micronutrients.

that are cancer promotors (eg, high fat intake), it may result in higher production of diacylglycerol. High concentrations of diacylglycerol promote a higher activation of protein kinase C, which can activate to a higher degree the *WNT* pathway, thereby promoting cancer growth.

In the present study, we also showed that carriers of the mutated allele benefited the most from a high calcium intake. It is known that APC protein also interacts with the transmembranar protein, E-cadherin, another cellular adhesion molecule (34). Because E-cadherin is a calcium-dependent protein, we can

speculate that a high concentration of calcium may strengthen these intercellular junctions, thereby inhibiting malignant transformation and growth. The same was observed in respect to fiber intake. A high intake of fiber would result in higher concentrations of butyrate. Butyrate is considered the first energy source for colonocytes, because it stimulates normal cell growth but inhibits tumor development, mainly by promoting cell apoptosis (35). These antiproliferative and proapoptotic effects of fiber consumption could somehow be amplified by the *D1822V* trait.

With the completion of the Human Genome Project, great opportunities exist to investigate the effects of genetic variation in the host on the use and metabolism of nutrients. To our knowledge, this is the first study performed in Southern Europe showing positive interactions between well-known dietary promoters and protectors of colorectal carcinogenesis and a polymorphism in the *APC* gene. The ultimate goal of this investigation will be to enable the provision of more precise public health advice about dietary intake, supplement use, and genetic testing.

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