Genetic analysis of Caveolin-1 and eNOS genes in colorectal cancer

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Abstract. Caveolae are involved in physical compartmentalization between different groups of signaling events. Its main component, CAV1, modulates different pathways in cellular physiology. The emerging evidence pointing to the role of CAV1 in cancer led us to study whether different alleles of this gene are associated with colorectal cancer (CRC). Since one of the most characterized enzymes regulated by CAV1 is eNOS, we decided to include both genes in this study. We analyzed five SNPs in 360 unrelated CRC patients and 550 controls from the general population. Two of these SNPs were located within eNOS and three within the CAV1 gene. Although haplotype distribution was not associated with CRC, haplotype TiA (CAV1) was associated with familiar forms of CRC ($p<0.05$). This was especially evident in CRC antecedents and nuclear forms of CRC. If both CG (eNOS) and TiA (CAV1) haplotypes were taken together, this association increased in significance. Thus, we propose that CAV1, either alone or together with eNOS alleles, might modify CRC heritability.

Introduction

Caveolae are lipid 'rafts' that form detergent-resistant microdomains (50-100 nm in diameter) in the cellular membrane. These structures are enriched in glycosphingolipids, sphingolipids and cholesterol, together with lipid-modified signaling molecules such as G-proteins, Src-tyrosine kinases and eNOS (1). Caveolae were traditionally associated to different cellular events such as transcytosis and endocytosis, but now their role in signal transduction is not in doubt. These microdomains exert a physical compartmentalization between different groups of signaling events, separating functionally unrelated signaling pathways and, therefore, facilitating cross-talk between related signaling modules (2-4). The proteins responsible for these structures, caveolins-1, -2 and -3, belong to a family of highly conserved integral membrane proteins. Caveolin-3 has been found only in muscle tissue (5), whilst caveolin-1 and -2 are ubiquitously expressed. Caveolin-1 (CAV1) encodes for a small protein (22 kDa) that constitutes the major component of the caveolae. CAV1 has been found to be necessary and sufficient to form the caveolae both *in vitro* and *in vivo* (6). The role of caveolins in different pathologies such as diabetes, muscular dystrophy, Alzheimer and cancer has been widely investigated (7-9).

Some authors have found a differential display of CAV1 in colon cancer cell lines and experimental colon adenocarcinomas when compared to normal tissue (10,11). However, the role of CAV1 in the aberrant cellular physiology is not fully understood. Caveolin-1 gene maps 7q31.1, a frequent unstable region within tumors with a high likelihood of LOH (12,13). There is also evidence that caveolin-1 promoter can be aberrantly methylated in pre-cancerous stages of colorectal cancer, which might be associated with its silencing during tumor development (14). Therefore, caveolin-1 has been proposed to be a tumor suppressor gene (13). However, there is also evidence suggesting a potential role of CAV1 as an oncogene under some circumstances (11,15,16).

One of the enzymes negatively regulated by caveolin-1 is the endothelial nitric-oxide synthase. eNOS is a large (133 kDa) lipid-modified protein found in the caveolae interacting with CAV1 (17,18). As a consequence, the eNOS catalytic activity is inhibited, preventing nitric oxide (NO) production. NO is a pleiotropic regulator, pivotal to multiple biological processes including vasodilation, neurotransmission or immunity. Furthermore, progression of a large majority of human tumors appears to be influenced by NO levels (19).

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Table I. Primers, probes and PCR conditions used for genotyping.[a]

Reference	PCR primers (5'-3')	PCR conditions	Pyrosequencing and FRET primers (5'-3')
eNOS			
rs1799983	CACAGCTCTGCATTCAGCAC	1. 5' at 95˚C,	Cy5-CTCTTCCTTCTGCCCCCCGAGCTGGTCC-P
(Glu298Asp)	TCCATCCCACCCAGTCAATC	2. [30"-95˚C, 30"-58˚C, 30"-72˚C]x50	CCCCAGATGATCCCCCAGA-FL
		3. 5' at 72˚C.	
rs2070744	TGCCTGGAGAGTGCTGGTGT-	1. 5' at 95˚C,	GGCTGAGGCAGGGTC
(T786C)	CGATCAGCAGAGAGACTAGG	2. [30"-95˚C, 20"-62˚C, 30"-72˚C]x50	
		3. 5' at 72˚C.	
CAV1			
rs3840634	AGACCTGCTTCGGGTCTGCT	1. 5' at 95˚C,	Cy5-AATGTGTGTACCCATTTCAG-P
(del)	Biotin-GGTCATCCATGACTGCCCTG	2. [30"-95˚C, 30"-68˚C, 30"-72˚C]x50	GGATCATTTCCCATTATACACTCA-FL
		3. 5' at 72˚C.	
rs3807990	AGACCTGCTTCGGGTCTGCT	1. 5' at 95˚C,	GTAGGAATCCTCTGCTC
(C>T)	Biotin-GGTCATCCATGACTGCCCTG	2. [30"-95˚C, 30"-68˚C, 30"-72˚C]x50	
		3. 5' at 72˚C.	
rs6867	CCATTGTGTGAGCCTATCAG	1. 5' at 95˚C,	GTAAAGCACTTGCAACC
(G>A)	Biotin-ACGACTCAGGTTTACAGGTG	2. [30"-95˚C, 30"-60˚C, 30"-72˚C]x50	
		3. 5' at 72˚C	

[a]FRET probes were phosforylated in 3' (P) or labeled with fluorescein (FL).

Recent studies have found that a high expression of eNOS in peritumoral microvessels protects against coloractal tumor metastasis (20). Moreover, evidence can also be found in the literature associating eNOS alleles with different parameters concerning ovarian and vulvar cancers (21,22). In particular, polymorphisms rs2070744 (also known as T786C) and rs1799983 (also named Glu298Asp), have been previously shown to be functional or, at least associated to other pathologies (23-28). Based on these observations, we decided to include both eNOS and CAV1 in the study of our colon adenocarcinoma series. Our aim was to elucidate whether these genes are involved in either the susceptibility or a differential prognosis of the disease.

We studied a total of 910 subjects, consisting of 360 unrelated colorectal cancer (CRC) patients and 550 controls from the general population. We studied whether CAV1 or eNOS is involved in CRC aetiology. Taken into account the studies aforementioned, we selected three single nucleotide polymorphisms (SNPs) within caveolin-1 (rs3807990, rs3840634 and rs6867) as well as two well-characterized SNPs of eNOS (rs2070744 and rs1799983) and compared their distribution between cases and controls.

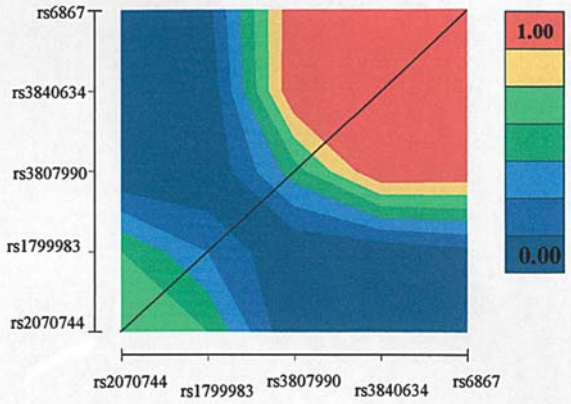
Materials and methods

Colorectal cancer cases and controls. A total of 360 cases and 550 controls were included (1:1.5 ratio). All subjects supplied written informed consent, together with a blood sample and a complete questionnaire regarding individual, environmental, familial variables, and pharmacological treatments. Only patients with confirmed histological diagnosis were included in our case series. We considered CRC-antecedent when at least one first- or second-degree relative also presented CRC. Aggregation indicates at least three independent cases of CRC confirmed among the first- or second-degree relatives. Nuclear cancer was defined when at least one first-degree relative was also diagnosed for CRC.

DNA extraction. We obtained 5 ml of peripheral blood from all patients to isolate germline DNA from leukocytes. DNA extraction was performed automatically according to standard procedures using Magnapure DNA isolation system (Roche, Germany). To perform polymerase chain reactions (PCRs), we prepared aliquots of DNA at a concentration of 5 ng/μl. The rest of the stock was cryopreserved at -20˚C.

Pyrosequencing and FRET assays. Primers and PCR conditions are summarized in Table I. PCR reactions were performed at a final volume of 20 μl, containing 5 ng of genomic DNA, 1.5 mM $MgCl_2$, 0.125 mM dNTPs, 2U of Taq, and 5 pmol of each primer. Amplification cycles were repeated 50x in order to abolish the free biotinylated primers that may interfere with the pyrosequencing reaction. For the SNPs located in CAV1 intron 2, PCR was performed at a final volume of 30 μl. After amplification, 10 μl were separated and rs3840634 (2-bp deletion) was analyzed using Light Cycler technology by means of fluorescence resonance energy transfer (FRET). On the other hand, rs3807990 (C>T) was determined by pyrosequencing using the remaining 20 μl. As an internal control, 10% of all DNA samples were independently re-extracted from blood and re-typed for each marker. Genotype concordance reached 98%. Unreliable genotypes derived from low-quality DNAs and inconsistent clinical data were discarded from the statistical analysis.

A)



B)

	rs1799983	rs3807990	rs3840634	rs6867
rs2070744	0.55 (<0.001)	0.04 (0.397)	-0.13 (0.086)	-0.04 (0.581)
rs1799983	-	0.05 (0.251)	-0.12 (0.181)	-0.02 (0.809)
rs3807990	-	-	-1.00 (<0.001)	0.96 (<0.001)
rs3840634	-	-	-	-1.00 (<0.001)

C)

	rs1799983	rs3807990	rs3840634	rs6867
rs2070744	0.59 (<0.001)	-0.06 (0.479)	0.16 (0.062)	-0.06 (0.511)
rs1799983	-	-0.15 (0.118)	0.04 (0.553)	-0.16 (0.149)
rs3807990	-	-	-1.00 (<0.001)	1.00 (<0.001)
rs3840634	-	-	-	-1.00 (<0.001)

Figure 1. Linkage disequilibrium between the SNPs under study in the entire population (A). D' calculated specifically for CRC cases with an associated p-value in brackets (B). D' calculated for controls, with the corresponding p-value in brackets (C).

Statistical analysis. The estimation of power to detect risk or protection to CRC was calculated for each SNP using Episheet sofware according to Rothman and Roice (29). For statistical analysis of genotype distribution, test for deviation of Hardy-Weinberg equilibrium or two-point association studies, we employed tests adapted from Sasieni (30). These calculations were performed using the online resource at the Institute for Human Genetics, Munich, Germany (http://ihg.gsf.de). To compare allele frequencies between groups, Chi-square tests with Yates correction or Fisher exact test were also performed according to Sasieni algorithms-based software, and confirmed using Statcalc (EpiInfo 5.1, Center for Disease Control, Atlanta, GA). Haplotype analysis was performed using the Thesias software (http://www.genecanvas.org) (31). This method allows the estimation of haplotype frequencies and haplotype effects by comparing with a reference (the intercept), taken here as the most conserved one. Haplotype effects are expressed as increases/decreases in the phenotype mean with respect to that of the intercept. To perform endocohort analysis, multiple testing correction using Bonferroni adjustment were performed (p-threshold: 0.0014). Graphical representations of LD were performed using GOLD 1.0, according to Abecasis *et al* (32).

Results

Single polymorphism analyses. Phenotypic characteristics of the study population are shown in Table II. As a first approach, we assessed the independent effect of age and sex on the risk of developing CRC. We found an increased risk in males [OR=1.31, p=0.055, 95% CI (0.99-11.78)], as previously reported (33). Increasing age was associated with greater risk of CRC. Thus, individuals aged between 61 and 70 years had an OR of 5.67 (p<0.001) when compared to those under 40 years. The corresponding estimate for ≥70 years was OR=11.07 (p<0.001). Gender, percentage of smokers, and body mass index (Kg/m^2), was similar in both groups. Cases and controls were all caucasian from central and southern Spain.

Genotypes at all loci for cases and controls were in Hardy-Weinberg equilibrium (Table III). Pairwise linkage disequilibrium (± D') between eNOS and CAV1 polymorphisms were also calculated for both cases and controls, either together or independently (Fig. 1). For CAV1, we detected a complete disequilibrium between rs3807990 and rs3840634, and also between rs6867 and rs3840634 (D'= -1, p< 0.001). Almost complete LD was also observed for CAV1 markers rs3807990 and rs6867 (D'=0.98, p<0.001). On the other hand, both eNOS polymorphisms (rs2070744 and rs1799983) showed a partial LD (D'=0.61, p<0.001). Both genes map to 7q and, according to different databases available, the physical distance between eNOS and CAV1 is 34 Mb. Thus, partial linkage disequilibrium was observed between those SNPs of eNOS and CAV1 in accordance with physical distance between the loci (Fig. 1A). When determined separatedly, D' values did not differ between cases and controls (Fig. 1B and C, respectively).

For eNOS variants, allele frequencies from rs2070744 (also named T786C) and rs1799983 (Glu298Asp) were in accordance with those previously reported for different European populations (0.47 and 0.38, respectively) (26-28). Regarding CAV1, this is the first study to our knowledge showing allele frequencies of the three SNPs under study [0.25 for rs3807990 (T), 0.19 for rs3840634 (d) and 0.22 for rs6867 (A)]. When allele frequencies were compared between cases and controls, no statistical difference was found (data not shown). Thus, it seems that at least in our study population, eNOS and CAV1 markers do not correlate with an increased risk of developing CRC.

On average, CRC was diagnosed at 64 years, ranging from 24 to 89 years. Thus, we performed quantitative regression analysis taking into account each SNP together with the age at CRC diagnosis. No association was found (data not shown). However, we are aware of the limited sample size which is especially relevant when quantitative trait loci are assayed. On the other hand, we performed endocohort studies

Table II. Clinical characteristics of study subjects.

	Controls (n=550)	Cases (n=360)
Age (years, mean ± SD)	55±12	67±11
Gender (male)	46%	54%
Smoker (ever)	47%	51%
Body mass index (Kg/m^2) (mean ± SD)	27±4.1	27±4.7
Family history (n=320)		
CRC antecedents	-	18.7%
CRC aggregation	-	4.3%
Nuclear CRC	-	17.1%
Cancer antecedents	-	54.3%
Cancer aggregation	-	17.5%
Nuclear cancer	-	50%

Table III. Genotype distribution between cases and controls.

Gene	SNP reference	Genotype	Cases	HWE	Controls	HWE
eNOS	rs2070744	CC	77	0.897	122	0.977
	(T786C)	TC	184		273	
		TT	107		152	
	rs1799983	GG	135	0.287	216	0.1
	(Glu298Asp)	TG	160		235	
		TT	60		87	
CAV1	rs3807990	CC	206	0.848	301	0.139
	(C>T)	CT	132		219	
		TT	20		28	
	rs3840634	ii	240	0.946	360	0.901
	(ins/del)	id	113		171	
		dd	13		21	
	rs6867	GG	223	0.556	318	0.498
	(G>A)	GA	101		183	
		AA	14		22	

to determine if eNOS and CAV1 were modifier factors of CRC. In this way, we analyzed whether these CAV1 or eNOS alleles modified CRC heritability. Among the CRC population, almost 19% showed familiar antecedents of CRC (Table II). In most cases (17%), these were a nuclear form of the disease (≥1 case in 1st-degree) whilst only 4% displayed an aggregation form of CRC (≥3 cases in 1st or 2nd-degree relatives). As can be seen in Table II, CRC has an important familiar component. In our series, a high percentage of the patients showed CRC antecedents. This percentage reached 54% when each malignant cancer was computed.

Some variants displayed a positive association with the familiar manifestations of CRC (Table IV). This was especially evident for CAV1 rs3807990 (C>T), although rs6867 was also associated to familiar forms of CRC. In contrast, neither of the SNPs of eNOS were associated to a family history of CRC. However, a trend was observed in eNOS rs2070744 [OR=1.49, 95% CI (0.97-2.3)]. The strong association between the CAV1 rs3807990 allele C, over-represented in all nuclear [OR=1.77, 95% CI (1.1-2.8), p=0.02], aggregation [OR=2.22, 95% CI (0.9-5.3), p=0.06], and familiar forms of CRC [OR=1.92, 95% CI (1.2-3.05), p=0.004]. However, in endocohort studies the appropriate control group is formed by those CRC cases that lack the parameter under study. This division significantly reduces the sample size. Consequently, these results should be considered with caution. Following this analysis, in order to get further insight into the influence of eNOS and CAV1 on CRC heritability we repeated these tests once the gene haplotypes were defined.

Haplotype analysis. Using Thesias software (31) and the data from those samples with no genotyping failure (n=830), we defined the CAV1 and eNOS haplotypes. For haplotype frequency determination, cases and controls were treated together. According to the resulting LD matrix, five haplotypes for CAV1 were represented in our series, and the four possible haplotypes for eNOS were present. Haplotypes with a prevalence <5% were not included in the study according to Tregouet *et al* (32). When haplotype frequencies were compared between cases and controls, no statistical difference was observed (data not shown). Again, using haplotype analysis we obtained evidence that eNOS and CAV1 alleles are not associated with an increased risk of developing CRC.

The lack of association to CRC predisposition did not exclude the possibility of correlation with a different heritability of CRC. To test this hypothesis, haplotype frequencies were analyzed together with the age of CRC onset and the different factors regarding colon cancer familiar antecedents, or any other cancer antecedents. Quantitative analysis of both eNOS and CAV1 haplotypes did not correlate with a earlier onset of the disease. On the other hand, CAV1 haplotype TiA showed an increased prevalence among the familiar forms of CRC (Table V). However, this observation did not reach statistical significance using SEM algorithm (p≥0.3). Conversely, when two-side cross tabulation analysis was performed comparing those patients with haplotype TiA versus the rest of the cases, the TiA haplotype was significantly associated with nuclear CRC (OR=1.83, p=0.036) and CRC antecedents (OR=1.84, p=0.02).

We also postulated that low penetrance alleles of eNOS and CAV1 might be genetically interacting among CRC patients. Therefore, we grouped both eNOS and CAV1 and analyzed their prevalence between cases and controls. Alternatively, we performed the same endocohort studies taking into account both genes in order to detect putative synergic effects. Surprisingly, the combination of two haplotypes: CG for eNOS, together with TiA (for CAV1) was significantly over-represented in nuclear CRC (4% vs. 12%, OR=4.4, p=0.008), CRC antecedents (4% vs. 12%, OR=4.2,

Table IV. Endocohort analysis of the CRC series.

	CAV1								
	rs3807990 (C>T)			rs3840634 (i/d)			rs6867 (G>A)		
Family history	Controls	Cases	p-value	Controls	Cases	p-value	Controls	Cases	p-value
Nuclear CRC	0.77	0.66	0.02	0.80	0.80	0.95	0.81	0.72	0.05
CRC aggregation	0.76	0.59	0.06	0.80	0.85	0.53	0.80	0.83	0.79
CRC antecedents	0.78	0.65	0.004	0.80	0.80	0.99	0.82	0.71	0.02
Nuclear cancer	0.77	0.75	0.59	0.80	0.79	0.74	0.80	0.80	0.96
Cancer aggregation	0.77	0.74	0.45	0.80	0.80	0.97	0.80	0.80	0.94
Cancer antecedents	0.76	0.75	0.87	0.80	0.80	0.91	0.80	0.80	0.96

	eNOS					
	rs2070744 (T786C)			rs1799983 (Glu298Asp)		
Family history	Controls	Cases	p-value	Controls	Cases	p-value
Nuclear CRC	0.46	0.48	0.77	0.61	0.62	0.72
CRC aggregation	0.47	0.42	0.62	0.60	0.71	0.31
CRC antecedents	0.47	0.47	0.90	0.60	0.63	0.54
Nuclear cancer	0.46	0.47	0.89	0.61	0.61	0.92
Cancer aggregation	0.48	0.38	0.06	0.60	0.65	0.21
Cancer antecedents	0.47	0.46	0.69	0.60	0.62	0.60

p=0.0061) and CRC aggregation (4% vs. 14%, not significant). Thus, we cannot exclude that both eNOS and CAV1 haplotypes might be genetically interacting among the familiar forms of CRC.

Discussion

Colorectal cancer is one of the most common neoplasms and a leading cause of death worldwide. Therefore, great investment has been made in order to gain new insight into its aetiology and how environmental and genetic factors influence the risk, onset and course of this malignancy (33,34). In the present study, we have used a genomic approach to study the involvement of caveolin-1 and eNOS in CRC. The emerging evidence pointing to the role of CAV1 in cancer, together with its regulatory properties over eNOS, led us to speculate on a digenic model for CRC progression. Following this hypothesis, we studied the allele frequency distribution of five SNPs within eNOS and CAV1 in CRC cases and the general population. The eNOS polymorphism rs1799983 encodes for a missense variant in eNOS exon 7 (Glu>Asp). This is a common SNP associated with a variety of diseases such as hypertension, pre-eclampsia, placental abruption or myocardial infarction (35-37). On the other hand, eNOS rs2070744 is a C>T conversion within intron 4 and it also has been studied in ovarian cancer and renal diseases (22,38). The wide range of phenotypes displayed by these variations proves the involvement of nitric oxide in multiple pathways, including carcinogenesis and tumor progression (39). However, our study firmly supports that there is no association between these eNOS polymorphisms and the susceptibility of CRC in the Spanish population.

Whenever negative associations are reported, the power of the study is questioned. However, we must state that according to the patient/control ratio, sample size and the allele prevalence for each marker, we should be able to detect positive OR ≥1.5 with a power of 80% and ≥1.6 in a more conservative fashion (90%) (Table VI). Therefore, the effect of these eNOS and CAV1 alleles on CRC risk, if present, would be very low. We found evidence of association between eNOS rs2070744 and cancer aggregation (O.R.=1.49, p=0.06). When eNOS haplotype distribution was studied in this sub-group, the -T background (CT+GT) was also significantly increased in the families with cancer aggregation (OR=2.9, p=0.022). However, this association did not support a more conservative analysis and multiple testing corrections (data not shown).

We studied the prevalence of three SNPs within CAV1 in our study population. The rationale for this study was that some authors found caveolin-1 overexpressed in experimental colon adenocarcinoma and its expression in human colon cancer cell lines directly correlates with their growth rate (11). Some studies with tissues from human prostate, breast and colon adenocarcinoma also showed overexpression of CAV1 (15,16). In contrast, previous reports using xenographs in nude mice showed that tumor formation *in vivo* resulted in the selection of cells with lower basal levels of caveolin-1 (10). Moreover, 7q31.1 is a frequently deleted region in different carcinomas, and tumor suppressor activity *in vitro* was reported (40,41). Thus, CAV1 has been considered a tumor suppressor gene (13). With this antecedents, it seemed necessary to circumvent the experimental designs and perform a genomic approach to elucidate the role of CAV1 in CRC. Following this approach, we were not

Table V. Haplotype frequencies within CRC cases.

	Nuclear CRC			CRC aggregation			CRC antecedents		
Haplotype	Controls	Cases	OR (p-value)	Controls	Cases	OR (p-value)	Controls	Cases	OR (p-value)
eNOS									
CT	0.31	0.33	1.2 (0.54)	0.32	0.22	0.77 (0.67)	0.32	0.32	1.1 (0.76)
CG	0.14	0.19	1.4 (0.28)	0.15	0.27	2.1 (0.30)	0.15	0.19	1.4 (0.36)
TT	0.09	0.09	1.2 (0.71)	0.09	0.13	1.6 (0.59)	0.09	0.09	1.1 (0.92)
TG	0.44	0.38	1.0[a]	0.43	0.37	1.0[a]	0.43	0.40	1.0[a]
CAV1									
CiG	0.57	0.45	0.7 (0.25)	0.55	0.45	0.79 (0.79)	0.57	0.44	0.68 (0.24)
CdG	0.19	0.21	1.0[a]	0.19	0.20	1.0[a]	0.19	0.21	1.0[a]
TiG	0.05	0.04	0.7 (0.59)	0.04	0.15	4.2 (0.16)	0.04	0.04	0.92 (0.89)
TiA	0.18	0.29	1.4 (0.37)	0.20	0.20	0.95 (0.94)	0.18	0.29	1.4 (0.3)
	Nuclear CRC			CRC aggregation			CRC antecedents		
Haplotype	Controls	Cases	OR (p-value)	Controls	Cases	OR (p-value)	Controls	Cases	OR (p-value)
eNOS									
CT	0.33	0.30	0.92 (0.71)	0.34	0.30	0.82 (0.36)	0.34	0.23	0.63 (0.15)
CG	0.15	0.16	1.2 (0.58)	0.15	0.16	1.0 (0.92)	0.15	0.16	0.97 (0.94)
TT	0.08	0.09	1.2 (0.59)	0.09	0.09	0.95 (0.9)	0.08	0.15	1.8 (0.15)
TG	0.43	0.43	1.0[a]	0.42	0.44	1.0[a]	0.42	0.45	1.0[a]
CAV1									
CiG	0.56	0.54	0.86 (0.50)	0.56	0.53	0.88 (0.65)	0.56	0.54	0.88 (0.60)
CdG	0.19	0.21	0.65 (0.38)	0.19	0.21	0.86 (0.82)	0.19	0.20	0.69 (0.45)
TiG	0.05	0.04	0.91 (0.71)	0.04	0.04	0.98 (0.97)	0.05	0.04	0.97 (0.92)
TiA	0.20	0.20	1.0[a]	0.20	0.21	1.0[a]	0.19	0.20	1.0[a]

[a]The haplotype taken as intercept.

Table VI. Minimum odds ratio detectable according to allele frequencies, sample size and the case/control ratio.

Gene	SNP reference	Frequency	Power 80%	Power 90%
eNOS	rs2070744 (C)	0.472	O.R. >1.5	O.R. >1.6
	rs1799983 (T)	0.385	O.R. >1.5	O.R. >1.6
CAV1	rs3807990 (T)	0.251	O.R. >1.6	O.R. >1.7
	rs3840634 (d)	0.192	O.R. >1.6	O.R. >1.7
	rs6867 (A)	0.217	O.R. >1.6	O.R. >1.7

able to find any direct association between rs3807990, rs3840634 or rs6867, and an altered likelihood of developing CRC. Therefore, it seems that these SNPs are not tumor markers in our population.

On the other hand, we detected a correlation between rs3807990 and CRC aggregation, nuclear forms and familiar antecedents of CRC (Table IV). In these groups, we found an over-representation of the allele T in all familial forms. In addition, rs6867 (G>A) was also associated with the familiar manifestations of CRC. Taken together, the results suggest that CAV1 alleles might modify CRC heritability. As expected, when haplotype distribution was studied, the prevalence of the TiA haplotype was increased among the risk groups. Moreover, the analysis performed taking eNOS and CAV1 together suggested an additive effect over both alleles. Further analysis should be done in order to confirm this hypothesis and to elucidate the true role that eNOS and CAV1 alleles on CRC aetiology.

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