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Genetic susceptibility in the development of colorectal adenomas according to family history of colorectal cancer

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Our study aimed to evaluate the relevance of genetic susceptibility in the development of colorectal adenomas (CRA) and its relationship with the presence of family history of colorectal cancer (CRC). Genomic DNA from 750 cases (first degree relatives of patients with CRC) and 750 controls (subjects with no family history of CRC) was genotyped for 99 single nucleotide polymorphisms (SNPs) previously associated with CRC/CRA risk by GWAS and candidate gene studies by using the MassArray™ (Sequenom) platform. Cases and controls were matched by gender, age and histological lesion. Eight hundred and fifty-eight patients showed no neoplastic lesions, whereas 288 patients showed low-risk adenomas, and 354 patients presented high-risk adenomas. Two SNPs (rs10505477, rs6983267) in the CASC8 gene were associated with a reduced risk of CRA in controls (log-additive models, OR: 0.67, 95%CI:0.54–0.83, and OR:0.66, 95%CI:0.54–0.84, respectively). Stratified analysis by histological lesion revealed the association of rs10505477 and rs6983267 variants with reduced risk of low- and high-risk adenomas in controls, being this effect stronger in low-risk adenomas (log-additive models, OR:0.63, 95% CI:0.47–0.84 and OR:0.64, 95%CI:0.47–0.86, respectively). Moreover, 2 SNPs (rs10795668, rs11255841) in the noncoding LINC00709 gene were significantly associated with a reduced risk of low-risk adenomas in cases (recessive models, OR:0.22, 95%CI:0.06–0.72, and OR:0.08, 95%CI:0.03–0.61) and controls (dominant models, OR:0.50, 95%CI:0.34–0.75, and OR:0.52, 95%CI:0.35–0.78, respectively). In conclusion, some variants associated with CRC risk (rs10505477, rs6983267, rs10795668 and rs11255841) are also involved in the susceptibility to CRA and specific subtypes. These associations are influenced by the presence of family history of CRC.

Key words: single nucleotide polymorphisms, colorectal adenoma, first degree relatives, colorectal neoplasia

Abbreviations: ASA: acetylsalicylic acid; CI: confidence interval; CRC: colorectal cancer; FDR: first degree relative; GWAS: genome-wide association studies; HRA: high-risk adenoma; LD: linkage disequilibrium; LRA: low-risk adenoma; NSAIDs: nonsteroidal anti-inflammatory drugs; OR: odds ratio; SD: standard deviation; SDR: second degree relative; SNP: single nucleotide polymorphism

Additional Supporting Information may be found in the online version of this article.

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What's new?

While numerous candidate gene variants have been associated with colorectal cancer, little is known about the relevance of genetic susceptibility or influence of family history in the development of precancerous colorectal adenomas. In the present study, certain genetic variants previously associated with colorectal cancer risk, including two variants in the *CASC8* gene and two in the lnc-RNA *LINC00709* gene, were found to be also involved in susceptibility to colorectal adenomas. The associations were modified by family history of colorectal cancer. The results could have implications for colorectal cancer screening and the identification of individuals at increased risk of colorectal adenoma.

Introduction

Colorectal cancer (CRC) is a major cause of morbidity and mortality throughout the world. Although the average survival at 5 years is close to 90% in early stages of the disease, less than 40% of CRC cases are diagnosed in these stages.[1] It is well known that CRC develops from premalignant colorectal lesions that require years to progress to invasive disease. Adenomas are the most common premalignant lesions and it is estimated that 70–90% of all CRC arise from colorectal adenomas. Epidemiological studies have confirmed that removal of adenomas sharply reduces the mortality from CRC.[2–4] As a result, many countries have launched in the past few years screening programs to detect precancerous lesions in asymptomatic individuals or adenocarcinomas at early stages of the disease.[5]

In this context, a great progress in understanding the genetic factors involved in the susceptibility to CRC has been made in the last two decades. Numerous candidate gene analysis[6,7] and genome-wide association studies (GWAS)[8–23] have identified a number of genetic variants, mainly single nucleotide polymorphisms (SNPs), associated with CRC risk. The risk conferred by each of these variants is usually modest. However, it has been observed that combination of risk variants in a polygenic model could increase the risk of CRC in an additive or exponential way.[24] These variants may have a special interest in the so-called nonsyndromic familial CRC. This type of CRC is generally defined by familiar aggregation of CRC to distinguish it from the well-established hereditary colorectal syndromes. Population-based studies estimated that approximately 20–25% of all CRC cases occur in first-degree relatives (FDRs) of patients with CRC. In fact, having a FDR with CRC has been reported to increase 2–4-fold the lifetime risk of developing CRC.[25–29] Taking into account that FDRs shares at least 50% of genes with a CRC patient in the same family (parents, offspring and siblings), it is rational to think that FDRs are more likely to present a coinheritance of multiple common variants in low penetrance genes that would provide them a greater risk of developing CRC than subjects with no family history of CRC.

Epidemiological studies have also reported an increased rate of colonic adenoma detection in individuals with family history of CRC compared to average-risk subjects.[30] Moreover, it has been shown that familial risk of colorectal adenomas is similar to familial risk of CRC, suggesting that some of the genetic predisposition to CRC conferred by common genetic variants may be mediated through increased adenoma risk. However, unlike the numerous studies performed in CRC, the relevance of genetic susceptibility in the development of colorectal adenomas and the influence of family history of CRC has been scarcely evaluated.

Trying to address these issues we design a case–control study to evaluate the role of certain SNPs associated with increased CRC risk in the development of colorectal adenomas according to the family history of CRC. In addition, we determined the relevance of these SNPs in the phenotypic expression of the lesion (low risk *vs.* high risk adenomas) according to the family history of CRC.

Material and Methods

Study population

This investigation was a case–control study with prospective data collection conducted in two general hospitals integrated into the Spanish National Health System. Subjects, cases and controls, were recruited at the University Hospital Lozano Blesa of Zaragoza and the University Hospital of the Canary Islands in Tenerife from May 2010 to May 2014.

As cases, we included 750 Spanish Caucasian FDRs of patients with nonsyndromic CRC selected from our CRC screening programs in Zaragoza and Tenerife. As controls we included 750 individuals with no family history of CRC matched by gender, age (±5 years) and histological lesions found during colonoscopy [non-neoplastic lesions, low risk adenomas (LRAs) and high risk adenomas (HRAs)]. Controls were recruited from those patients who were scheduled for colonoscopy either by symptoms or by CRC screening in the average- risk population. Exclusion criteria included: hereditary CRC syndromes (hereditary nonpolyposis CRC or familial adenomatous polyposis), CRC or previous history of CRC, inflammatory bowel disease, prior polypectomy without pathology report of removed polyp, age < 18 years old, insufficient blood sample for SNPs analysis, lack of information on essential demographic variables, and ethnicity other than Caucasian.

All cases and controls underwent at least one colonoscopy. The following three groups were defined on the basis of the endoscopic findings and the standardized pathology review: (1) patients with no lesions or with no neoplastic lesions, (2) patients with LRA, defined as <3 nonadvanced adenomas and (3) patients with HRA, defined as advanced adenomas or ≥ 3 nonadvanced adenomas. This stratification is based on the likelihood of developing advanced neoplasia during surveillance

after polypectomy as recommended by the European and American Societies of Gastrointestinal Endoscopy.[4–31] Adenomas were classified as advanced if they were ≥ 10 mm or/and had ≥20% villous components or/and high grade dysplasia. If a patient had undergone several colonoscopies, the colonoscopy with the most advanced lesion was included in the study. The rate of complete colonoscopies was high in both, cases (99.1%) and controls (97.7%). Similarly, the quality of preparation for colonoscopy was good or very good (≥ 6 in Boston scale) in cases (86.1%) and controls (88.4%) and only 2.5% of subjects showed a deficient preparation.

Participants were interviewed with a structured questionnaire administered by trained personnel. Information regarding demographic characteristics and potential factors affecting the risk of colorectal neoplastic lesions such as family history of CRC (any reported CRC in FDR or two or more CRC cases in second-degree relatives), smoking habit (never, former or current), alcohol intake, and chronic use of nonsteroidal anti-inflammatory drugs (NSAIDs) or low-dose (≤ 300 mg) of acetylsalicylic acid (ASA) were obtained. According to the World Health Organization, nondrinkers were defined as patients taking ≤1 drink (10 g of alcohol)/weekly. Regarding tobacco, current smoker was defined as someone smoking ≥100 cigarettes (including hand rolled cigarettes, cigars, etc.) in his lifetime and who currently smokes. Former smoker was defined as someone smoking ≥100 cigarettes in his lifetime but had quit smoking at the time of interview. Some other variables related to the quality of colonoscopy (cecal intubation and bowel preparation), and characteristics of the lesion (number, size, location and histology, including degree of dysplasia) were also collected.

After completion of the interview, 10 mL of peripheral blood from each subject was collected for DNA extraction. Genomic DNA was extracted from ethlyenediaminetetraacetic acid (EDTA)-preserved whole blood in an AutoGenFlex 3000. DNA samples were aliquoted and stored at 4 °C until analysis.

All participants gave written informed consent to the study which was conducted in accordance with the Ethical Committee of the Hospitals.

SNP Selection and Genotyping

The panel of polymorphisms included in our study was selected *a priori* from the NCBI data base (http://www.ncbi.nlm.nih.gov/snp) and the NHGRI-EBI GWAS Catalog (http://www.ebi.ac.uk/gwas) based on three main criteria: (1) published evidence of an association with CRC or CRA risk by GWAS o candidate gene studies; (2) having reported a prevalence of at least 1% for the less frequent allele among Caucasians or (3) having potential functional consequences leading to altered protein concentrations or protein functions.

Finally, a total of 99 SNPs previously reported to be associated with CRC/CRA risk were consider for analysis (Supporting Information Table 1). Genotyping was performed at the Spanish National Genotyping Centre (CEGEN-Santiago de Compostela) using the Sequenom MassARRAY iPLEX platform. As a quality control, 5% of samples, including internal controls by Spanish National Genotyping Centre, were analyzed in duplicated with a concordance rate of 100% for all assays. Among the 99 SNPs analyzed, 11 SNPs were excluded from the study due to failure of genotyping (rs11632715, rs17730929, *PTGS1* rs3842787 and *PNMAL1* rs7248888), SNP call rate < 90% (*TPH2* rs10879357, *MYRF* rs174537, *PTGS2* rs20417, *ERCC2* rs1799793 and *HADC9* rs1919314) or deviation from Hardy–Weinberg equilibrium among controls (Fisher's test $p < 10^{-4}$, rs11671104, rs2965667). In our study, genotype completion on genomic DNA samples exceeded 99%. Finally, 88 SNPs in 1,500 subjects (750 cases and 750 controls) were successfully genotyped and available for analysis.

Statistical analysis

An initial exploratory analysis of all clinical variables was carried out. Continuous variables were expressed as mean with standard deviation (SD) whereas qualitative variables were expressed as frequencies and percentages. The relationship between qualitative variables was evaluated with Chi-square (χ^2) test. Student *t*-test or Mann–Whitney *U* test were employed for comparing means of two independent groups. Normality was tested using Kolmogorov–Smirnov test.

Regarding the study of SNPs, genotype frequencies for each polymorphism among controls were tested for Hardy–Weinberg equilibrium by a χ^2 test with one degree of freedom (df). Genotype and allele frequencies between cases and controls were compared using the χ^2 test with Yates' correction or Fisher's exact test. The magnitude of the association of each SNP with the response variable was estimated by Odds Ratio (OR) and 95% confidence interval (CI) using the *SNPassoc* package implemented in R 3.2.2. Analyses were performed using codominant, dominant, recessive, overdominant, and log-additive genetic models. Finally, the influence of genetic factors in the development of premalignant lesions was assessed using logistic regression analysis adjusting by gender, age, family history of CRC, consumption of tobacco, alcohol, NSAIDs, and low-dose ASA. A two-sided *p*-value <0.05 was considered statistically significant. In order to address the problem of multiple comparisons, the Bonferroni correction and False Discovery Rate method were applied. Statistical analysis was performed using SPSS 22.0 (SPSS Ibérica, Madrid, Spain).

Taking into account the prevalence of the analyzed SNPs in our population, the size of the study was sufficient to detect ORs > 1.413 or < 0.727 with a power of 80% and an alpha value of 0.05. For the less prevalent polymorphisms (MAF: 0.02–0.10), the study had a power of 80% to detect an OR of >4.850 in the whole data set. All power calculations were performed using the programme Epidat 4.1.

Results

Clinical and demographic characteristics of patients

The clinical and demographic characteristics of cases (FDRs of patients with CRC) and controls (individuals without family

Table 1. Clinical and demographic characteristics of the study population

Clinical and demographic characteristics		Cases (n = 750) n (%)	Controls (n = 750) n (%)	p-value
Age	Mean (SD) Median (min – max)	54.4 (9.6) 55.0 (30–84)	54.7 (9.6) 55.0 (26–82)	0.285
Gender	Males	362 (48.3)	362 (48.3)	1.000
Alcohol	No	421 (56.1)	462 (61.6)	0.303
	Yes	291 (38.8)	286 (38.1)	
	Unknown	38 (5.1)	2 (0.3)	
Tobacco	Never smoker	393 (52.4)	408 (54.4)	0.499
	Current smoker	184 (24.5)	203 (27.1)	
	Former smoker	149 (19.9)	137 (18.3)	
	Unknown	24 (3.2)	2 (0.3)	
NSAIDs (chronic use)	Yes	43 (5.7)	57 (7.6)	0.147
	No	704 (93.9)	692 (92.3)	
	Unknown	3 (0.4)	1 (0.1)	
Low-dose ASA (chronic use)	Yes	34(4.5)	48 (6.4)	0.335
	No	621 (82.8)	702 (93.6)	
	Unknown	95 (12.7)	0 (0.0)	
Findings on	No lesions*	429 (57.2)	429 (57.2)	1.000
colonoscopy	LRA	144 (19.2)	144 (19.2)	
	HRA	177 (23.6)	177 (23.6)	
	CRC	0	0	

n: number of individuals. SD: standard deviation. NSAIDs: nonsteroidal anti-inflammatory drugs. ASA: acetylsalicylic. LRA: low risk adenoma. HRA: high risk adenoma. CRC: colorectal cancer.
*No colorectal neoplastic lesions.

history of CRC) are shown in Table 1. The average age of participants was 54.5 ± 9.4 years with a slight predominance of women (n = 776; 51.7%). No significant differences between cases and controls were observed regarding consumption of tobacco, alcohol, and chronic use of NSAIDs or low-dose ASA. Eight hundred and fifty-eight patients (57%) patients, cases and controls, had no neoplastic lesions, 288 patients (144 cases, 144 controls) had LRA and 354 patients (177cases, 177controls) had HRA. Of interest, patients with adenomas were significantly older than patients with no neoplastic lesions (average age 56 *vs.* 53.5, $p < 0.001$), showed a predominance for male gender (59.2% *vs.* 40.1%, $p < 0.001$) and reported significant higher consumption of tobacco (27.3% *vs.* 24.7%, $p = 0.021$), alcohol (45.5% *vs.* 33.2%, $p < 0.001$), and lower chronic use of NSAIDs (5.1% *vs.* 7.8%, $p = 0.043$).

Family history of CRC in the studied population

As mentioned, 50% of subjects included in our study (n = 750) and referred as cases, had at least one FDR affected with CRC. Most cases had 1 FDR with CRC diagnosed >60 years (63.6%, 477/750) or ≤ 60 years (25.6%, 192/750). Seventy nine patients (10.5%) had two FDR with CRC. Two patients had 1 FDR with CRC diagnosis at unknown age. Mean age at diagnosis of CRC in FDRs was 66 ± 12.6 years. Age at diagnosis was less than 60 years nearly 30% of index cases (patients with CRC). It should be noted that 20% of cases (151/750) had both, FDRs and second degree relatives (SDRs) with CRC. Parents were the most often affected FDRs (68.8%), followed by siblings (30.5%) and children (0.6%).

When considering the histological findings, we observed that cases with two FDRs with CRC were significantly more frequent in the group of patients with adenomas that in the group with no neoplastic lesions (14.3% *vs.* 7.9%, OR: 1.9, 95% CI: 1.2–3.1, $p = 0.005$). This difference was even greater in the subgroup of patients with HRA (17.5% *vs.* 7.9%, OR: 2.5, 95% CI: 1.5–4.2, $p = 0.001$).

Genotyping

Of the 99 SNPs initially selected in our study, 88 SNPs were successfully genotyped in 1,500 subjects (750 cases and 750 controls) and available for analysis. Supporting Information Table 2 summarizes the genotype distribution of each polymorphism in cases and controls. Genotype frequencies did not deviate significantly from those expected under Hardy–Weinberg equilibrium in the control group (Fisher's test $p > 10^{-4}$).

Gene polymorphisms and susceptibility to colorectal adenomas

Of the 88 SNPs included in the statistical analysis, 15 SNPs (rs10505477, rs11255841, rs11903757, rs13181, rs1330344,

Table 2. SNPs significantly associated with risk of colorectal adenomas in the study population

SNP (Gene)	Genetic model	Genotype	Normal n	Adenoma n	OR	95% CI		*p*-value[1]	FDR[2]
rs10505477	Recessive	A/A-A/G	653	436	1.00	Reference		0.014	**0.033**
CASC8		G/G	184	85	0.69	0.52	0.93		
rs6983267	Recessive	G/G-G/T	647	427	1.00	Reference		0.010	**0.033**
CASC8		T/T	175	77	0.67	0.50	0.91		
rs13181	Recessive	T/T-G/T	727	470	1.00	Reference		0.015	**0.033**
ERCC2		G/G	110	50	0.64	0.44	0.92		
rs1728785	Recessive	C/C-A/C	773	503	1.00	Reference		<0.001	**0.015**
ZFP90		A/A	62	18	0.41	0.24	0.72		
rs8180040	Recessive	T/T-A/T	686	456	1.00	Reference		0.003	**0.026**
PTPN23		A/A	150	66	0.62	0.45	0.86		
rs16260	Recessive	C/C-A/C	758	489	1.00	Reference		0.013	**0.033**
CDH1		A/A	79	34	0.59	0.38	0.91		
rs9929218	Recessive	G/G-A/G	756	489	1.00	Reference		0.005	**0.029**
CDH1		A/A	83	34	0.55	0.36	0.84		

OR: Odds ratio. CI: confidence interval. FDR: False Discovery Rate. n: number of individuals.
[1]ORs and p-values adjusted by age, gender, tobacco, alcohol, drugs use (NSAIDs and low-dose ASA), and family history of CRC.
[2]p-values obtained after applying the False Discovery Rate (FDR) test for multiple corrections. FDR values <0.05 are highlighted in bold. Only those models with significant FDR p-values are shown in the table.

rs16260, rs1665650, rs1728785, rs367615, rs4779584, rs6983267, rs8180040, rs9365723, rs961253 and rs9929218) were significantly associated ($p < 0.05$) with the presence of colorectal adenomas in at least one of five genetic models evaluated in the multivariate analysis (Supporting Information Table 3). After False Discovery Rate multiple test correction, seven SNPs located in the *CASC8* (rs10505477A>G, rs6983267G>T), *ZFP90* (rs1728785C>A), *ERCC2* (rs13181T>G), *PTPN23* (rs8180040T>A), and *CDH1* (rs9929218G>A, rs16260C>A) genes retained significance (recessive models) (Table 2).

Stratified analysis by family history of CRC revealed highly significant associations between the two intronic variants, rs10505477 and rs6983267 located in the *CASC8* (*cancer susceptibility candidate 8*) gene and lower risk of adenomas in the subgroup of patients with no family history of CRC (controls) (Table 3). Notably, these associations maintained significant values after applying False Discovery Rate and Bonferroni corrections (Supporting Information Fig. 1).

Besides *CASC8* rs10505477 and rs6983267, four additional SNPs were specifically associated with the risk of adenomas in patients with no family history of CRC after False Discovery Rate correction (Table 3). In this regard, the intronic variants rs10795668G>A and rs11255841T>A in the *LINC00709* gene, and the rs647161A>C in *C5orf66* showed a significant association with reduced risk of adenomas in patients with no family history of CRC. By contrast, the intergenic rs4779584 variant (*GREM1- SCG5*) was associated with a higher risk of developing colorectal adenomas (additive model, OR: 1.52; 95% CI: 1.15–2.02). Unlike controls, no significant associations with risk of adenomas were observed in FDRs of patients with CRC (cases) after False Discovery Rate multiple test correction (data not shown).

Gene polymorphisms and phenotypic expression of the lesion (LRA *vs.* HRA)

Subgroup analysis by type of adenoma (LRA *vs.* HRA) revealed some interesting associations. Table 4 summarizes those SNPs significantly associated with subtypes of adenomas in the overall population (cases and controls) after False Discovery Rate correction. The intronic variants *CASC8* rs10505477 and rs6983567 were associated with a lower risk of developing HRAs (additive models, OR: 0.77; 95% CI: 0.63–0.94 and OR: 0.78, 95% CI: 0.64–0.95, respectively). By contrast, the intergenic variant rs4779584 (*GREM1- SCG5*) was associated with an increased risk of HRAs (additive model, OR: 1.50; 95% CI: 1.17–1.92).

Stratified analysis by family history of CRC showed a significant association of *CASC8* rs10505477 and rs6983267 variants with decreased risk of both LRAs and HRAs in patients with no family history of CRC (controls) (Table 5). This protective effect was stronger on the risk of LRAs (recessive models, OR: 0.38; 95% CI: 0.21 to 0.67 for rs10505477 and OR: 0.32, 95% CI 0.17 to 0.61 for rs6983267). Notably, associations of both rs10505477 and rs6983267 with reduced risk of LRAs retained significant values after Bonferroni correction (Supporting Information Fig. 2). Besides *CASC8* variants, some other SNPs located in the long noncoding gene *LINC00709* (rs10795668 and rs11255841) and *CDH1* (rs16260 and rs9929218) genes were associated with a reduced risk of developing LRAs in patients with no family history of CRC. Similarly, to *CASC8* variants, *LINC00709* rs10795668 and rs1125584 maintained statistically significant

Table 3. SNPs significantly associated with risk of adenomas in patients without family history of CRC

SNP (Gene)	Genetic model	Genotype	Normal n	Adenoma n	OR	95% CI		*p*-value[1]	FDR[2]
rs10505477	Codominant	A/A	117	110	1.00	Reference		<0.001	**0.047**
CASC8		A/G	204	161	0.79	0.56	1.12		
		G/G	106	46	0.43	0.27	0.67		
		A/G-G/G	310	207	0.66	0.47	0.92		
	Recessive	A/A-A/G	321	271	1.00	Reference		<0.001	**0.003**
		G/G	106	46	0.49	0.33	0.73		
	Additive	AA,AG,GG	427	317	0.67	0.54	0.83	<0.001	**0.004**
rs6983267	Codominant	G/G	127	114	1.00	Reference		<0.001	0.065
CASC8		G/T	191	152	0.84	0.59	1.18		
		T/T	102	41	0.42	0.26	0.66		
		G/T–T/T	293	193	0.69	0.50	0.95		
	Recessive	G/G-G/T	318	266	1.00	Reference		<0.001	**0.003**
		T/T	102	41	0.46	0.31	0.70		
	Additive	GG,GT,TT	420	307	0.67	0.54	0.84	<0.001	**0.004**
rs10795668	Codominant	G/G	182	169	1.00	Reference		0.013	**0.042**
LINC00709		A/G	207	118	0.62	0.45	0.86		
		A/A	39	30	0.86	0.50	1.47		
		A/G-A/A	246	148	0.66	0.49	0.89		
	Overdominant	G/G-A/A	221	199	1.00	Reference		0.003	**0.027**
		A/G	207	118	0.64	0.47	0.87		
rs11255841	Codominant	T/T	196	181	1.00	Reference		0.015	**0.042**
LINC00709		A/T	201	112	0.63	0.46	0.87		
		A/A	30	23	0.93	0.51	1.70		
		A/T-A/A	231	135	0.67	0.49	0.91		
	Overdominant	T/T-A/A	226	204	1.00	Reference		0.003	**0.027**
		A/T	201	112	0.64	0.47	0.87		
rs4779584	Codominant	C/C	304	196	1.00	Reference		0.007	**0.037**
GREM1 -		C/T	112	109	1.68	1.20	2.34		
SCG5		T/T	11	12	1.60	0.67	3.86		
		C/T–T/T	123	121	1.67	1.21	2.31		
	Overdominant	C/C-T/T	315	208	1.00	Reference		0.003	**0.027**
		C/T	112	109	1.64	1.18	2.29		
	Additive	CC,CT,TT	427	317	1.52	1.15	2.02	0.003	**0.017**
rs647161	Codominant	A/A	166	160	1.00	Reference		0.011	**0.042**
C5orf66		A/C	199	128	0.69	0.50	0.95		
		C/C	63	30	0.52	0.31	0.86		
		C/C	63	30	0.62	0.39	1.01		
	Additive	AA,AC,CC	428	318	0.71	0.57	0.89	0.002	**0.017**

OR: Odds ratio. CI: confidence interval. FDR: False Discovery Rate. n: number of individuals.
Only those models with significant FDR p-values are shown in the table.
[1]ORs and p-values adjusted by age, gender, tobacco, alcohol and drugs use (NSAIDs and low-dose ASA).
[2]p-values obtained after applying the False Discovery Rate (FDR) test for multiple corrections. FDR values <0.05 are highlighted in bold.

associations after Bonferroni correction (overdominant models, OR: 0.47, 95% CI: 0.31–0.71 and OR: 0.48, 95% CI: 0.32–0.73, respectively) (Supporting Information Fig. 2). On the other hand, carriers of the *GREM1- SCG5* rs4779584T variant were at higher risk of presenting HRAs (dominant model, OR:1.92, 95% CI:1.31–2.83) (Table 5).

Concerning the subgroup of FDRs of patients with CRC (cases), Table 6 shows those SNPs significantly associated with

Table 4. SNPs associated with risk of adenoma subtypes in the overall population

SNP (Gene)	Genetic Model	Genotype	Adenoma		Low risk adenoma							High risk adenoma						
					No*	Yes**						No*	Yes**					
			p-value[1]	FDR[2]	n	n	OR	95%	CI	*p*-value[1]	FDR[2]	n	n	OR	95%	CI	*p*-value[1]	FDR[2]
rs10505477	Cod	A/A	**0.018**	0.128	234	75	1.00			0.085	0.237	234	95	1.00			**0.039**	0.070
CASC8		A/G			419	125	0.87	0.62	1.22			419	141	0.77	0.56	1.06		
		G/G			184	37	0.61	0.39	0.95			184	48	0.59	0.39	0.90		
	Rec	A/A-A/G	**0.014**	**0.034**	653	200	1.00			**0.039**	0.077	653	236	1.00			**0.047**	0.097
		G/G			184	37	0.66	0.44	0.99			184	48	0.70	0.48	1.00		
	Adi	AA,AG,GG	**0.005**	0.057	837	237	0.79	0.64	0.98	**0.034**	0.081	837	284	0.77	0.63	0.94	**0.011**	**0.039**
rs6983267	Cod	G/G	**0.020**	0.138	254	79	1.00			0.075	0.293	254	99	1.00			0.050	0.095
CASC8		G/T			393	116	0.91	0.65	1.28			393	133	0.81	0.59	1.12		
		T/T			175	33	0.60	0.38	0.95			175	44	0.60	0.39	0.91		
	Rec	G/G-G/T	**0.010**	**0.034**	647	195	1.00			**0.027**	0.076	647	232	1.00			**0.036**	0.097
		T/T			175	33	0.63	0.42	0.96			175	44	0.67	0.46	0.98		
	Adi	GG,GT,TT	**0.007**	0.057	822	228	0.80	0.64	0.99	**0.040**	0.081	822	276	0.78	0.64	0.95	**0.015**	**0.039**
rs4779584	Cod	C/C	**0.047**	0.128	569	162	1.00			0.300	0.979	569	167	1.00			**0.003**	**0.007**
GREM1		C/T			242	70	1.08	0.78	1.50			242	104	1.65	1.22	2.24		
SCG5		T/T			26	5	0.50	0.18	1.38			26	13	1.69	0.83	3.47		
	Dom	C/C	**0.019**	0.079	569	162	1.00			0.952	0.993	569	167	1.00			**<0.001**	**0.032**
		C/T–T/T			268	75	1.01	0.73	1.39			268	117	1.66	1.24	2.22		
	Over	C/C-T/T	**0.014**	0.105	595	167	1.00			0.543	0.977	595	180	1.00			**0.002**	**0.020**
		C/T			242	70	1.11	0.80	1.54			242	104	1.60	1.19	2.16		
	Adi	CC,CT,TT	**0.049**	0.070	837	237	0.94	0.71	1.25	0.682	0.972	837	284	1.50	1.17	1.92	**0.001**	**0.013**

OR: odds ratio. CI: confidence interval. FDR: False Discovery Rate. n: number of individuals. Rec: recessive genetic model. Dom: dominant genetic model. Cod: codominant genetic model. Adi: additive genetic model. Over: overdominant genetic model. *Number of patients without adenomas ** Number of patients with colorrectal adenomas.
Only those SNPs with significant FDR p-values are shown in the table.
[1]ORs and p-values adjusted by age, gender, tobacco, alcohol, drugs use (NSAIDs and low-dose ASA), and family history of CRC. p-values <0.05 are highlighted in bold.
[2]p-values obtained after applying the False Discovery Rate (FDR) test for multiple corrections. FDR values <0.05 are highlighted in bold.

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Table 5. SNPs associated with risk of adenoma subtypes in patients without family history of CRC (controls)

SNP (Gene)	Genetic Model	Genotype	Adenoma		Low risk adenoma							High risk adenoma						
					No*	Yes**						No*	Yes**					
			p-value[1]	FDR[2]	n	n	OR	95%	CI	*p*-value[1]	FDR[2]	n	n	OR	95%	CI	*p*-value[1]	FDR[2]
rs10505477	Cod	A/A	**<0.001**	**0.047**	117	48	1.00			**0.001**	0.133	117	62	1.00			**0.018**	0.073
CASC8		A/G			204	79	0.85	0.54	1.34			204	82	0.70	0.46	1.06		
		G/G			106	16	0.34	0.18	0.65			106	30	0.47	0.28	0.80		
	Rec	A/A-A/G	**<0.001**	**0.004**	321	127	1.00			**<0.001**	**0.003**	321	144	1.00			**0.022**	0.092
		G/G			106	16	0.38	0.21	0.67			106	30	0.59	0.37	0.94		
	Adi	AA, AG,GG	**<0.001**	**0.004**	427	143	0.63	0.47	0.84	**0.002**	**0.015**	427	174	0.69	0.53	0.89	**0.005**	**0.028**
rs6983267	Cod	G/G	**<0.001**	0.065	127	49	1.00			**<0.001**	0.179	127	65	1.00			**0.022**	0.085
CASC8		G/T			191	74	0.94	0.60	1.48			191	78	0.73	0.48	1.11		
		T/T			102	13	0.31	0.16	0.62			102	28	0.48	0.28	0.82		
	Rec	G/G-G/T	**<0.001**	**0.004**	318	123	1.00			**<0.001**	**0.002**	318	143	1.00			**0.019**	0.092
		T/T			102	13	0.32	0.17	0.61			102	28	0.57	0.36	0.92		
	Adi	GG,GT,TT	**<0.001**	**0.004**	420	136	0.64	0.47	0.86	**0.002**	**0.015**	420	171	0.70	0.54	0.90	**0.006**	**0.028**
rs10795668	Cod	G/G	**0.013**	**0.043**	182	85	1.00			**0.001**	**0.009**	182	84	1.00			0.430	0.765
LINC00709		A/G			207	44	0.45	0.29	0.69			207	74	0.78	0.53	1.14		
		A/A			39	14	0.79	0.39	1.57			39	16	0.93	0.48	1.79		
	Dom	G/G	**0.007**	0.054	182	85	1.00			**<0.001**	**0.006**	182	84	1.00			0.234	0.808
		A/G-A/A			246	58	0.50	0.34	0.75			246	90	0.80	0.55	1.15		
	Over	G/G-A/A	**0.004**	**0.028**	221	99	1.00			**<0.001**	**0.003**	221	100	1.00			0.201	0.685
		A/G			207	44	0.47	0.31	0.71			207	74	0.79	0.54	1.14		
	Adi	GG, AG, AA	0.051	0.108	428	143	0.67	0.49	0.92	**0.013**	**0.033**	428	174	0.88	0.66	1.18	0.391	0.784
rs11255841	Cod	T/T	**0.015**	**0.043**	196	90	1.00			**0.002**	**0.009**	196	91	1.00			0.458	0.765
LINC00709		A/T			201	42	0.47	0.31	0.73			201	70	0.79	0.54	1.16		
		A/A			30	11	0.86	0.40	1.86			30	12	1.01	0.48	2.11		
	Dom	T/T	**0.009**	0.054	196	90	1.00			**0.001**	**0.007**	196	91	1.00			0.279	0.808
		A/T-A/A			231	53	0.52	0.35	0.78			231	82	0.82	0.57	1.18		
	Over	T/T-A/A	**0.004**	**0.028**	226	101	1.00			**<0.001**	**0.003**	226	103	1.00			0.211	0.685
		A/T			201	42	0.48	0.32	0.73			201	70	0.79	0.55	1.14		
	Adi	TT, AT, AA	0.056	0.108	427	143	0.67	0.48	0.93	**0.016**	**0.034**	427	173	0.89	0.66	1.20	0.451	0.794
rs9929218	Rec	G/G-A/G	**0.033**	0.076	379	136	1.00			**0.012**	**0.034**	379	159	1.00			0.341	0.821
CDH1		A/A			49	7	0.38	0.16	0.87			49	16	0.75	0.40	1.38		
	Adi	GG, GA, AA	**0.040**	0.105	428	143	0.71	0.52	0.97	**0.027**	**0.044**	428	175	0.86	0.65	1.13	0.266	0.783
rs16260	Rec	C/C-A/C	**0.048**	0.080	379	136	1.00			**0.014**	**0.034**	379	159	1.00			0.418	0.835
CDH1		A/A			48	7	0.38	0.17	0.89			48	16	0.78	0.42	1.44		

rs4779584	Cod	C/C																
			0.008	**0.038**	304	93	1.00			0.317	0.808	304	103	1.00			**0.004**	**0.017**
GREM1 SCG5		C/T			112	46	1.38	0.90	2.12			112	63	1.88	1.26	2.81		
		T/T			11	4	0.85	0.24	2.93			11	8	2.31	0.87	6.11		
	Dom	C/C	**0.002**	0.053	304	93	1.00			0.192	0.831	304	103	1.00			**<0.001**	0.069
		C/T–T/T			123	50	1.32	0.87	2.02			123	71	1.92	1.31	2.83		
	Over	C/C-T/T	**0.003**	**0.028**	315	97	1.00			0.136	0.904	315	111	1.00			**0.004**	0.069
		C/T			112	46	1.39	0.91	2.13			112	63	1.80	1.21	2.67		
	Adi	CC,CT,TT	**0.003**	**0.017**	427	143	1.21	0.84	1.74	0.317	0.714	427	174	1.73	1.25	2.40	**0.001**	**0.021**

OR: odds ratio. CI: confidence interval. FDR: False Discovery Rate. n: number of individuals. Rec: recessive genetic model. Dom: dominant genetic model. Cod: codominant genetic model. Adi: additive genetic model. Over: overdominant genetic model. *Number of patients without adenomas ** Number of patients with colorrectal adenomas.
Only those SNPs with significant FDR p-values are shown in the table.
[1]ORs and p-values adjusted by age, gender, tobacco, alcohol, and drugs use (NSAIDs and low-dose ASA). p-values <0.05 are highlighted in bold.
[2]p-values obtained after applying the False Discovery Rate (FDR) test for multiple corrections. FDR values <0.05 are highlighted in bold.

LRAs or HRAs after False Discovery Rate correction. As observed in the group of patients with no family history of CRC (controls), the long noncoding *LINC00709* rs10795668 and rs11255841 variants were associated with a lower risk of LRAs in cases. Moreover, rs11255841 maintained significant associations with LRAs risk in several genetic models after Bonferroni correction (codominant model, OR:0.10; 95% CI:0.01–0.76; recessive model, OR:0.08, 95% CI 0.01–0.61) (Supporting Information Fig. 3). Thus, the protective effect of rs10795668 and rs11255841 variants in the development of LRAs was detected in both, cases and controls, regardless the presence of family history of CRC. Finally, two other SNPs located in the *XPC* (rs2228000G>A) and *CABLES2* (rs2427308C>T) genes were specifically associated with LRAs in FDRs of patients with CRC. The nonsynonymous rs2228000 SNP (Ala462Val) in the nucleotide excision repair gene *XPC* was associated with an increased risk of LRAs (additive model OR: 1.62, 95% CI 1.13–2.30) whereas the intronic *CABLES2* rs2427308 variant was associated with a decreased risk of developing LRAs (dominant model, OR: 0.59, 95% CI: 0.35–0.98). No risk variants were found to be associated with the susceptibility to HRAs.

Discussion

Over the last two decades, numerous association studies have been conducted in order to assess the relevance of common gene polymorphisms on CRC risk.[6–23] However, the influence of gene variants in the development of colorectal adenomas and the role of CRC family history in this association has been scarcely analyzed.

In our study, seven SNPs located in the *CASC8* (rs10505477 A>G, rs6983267G>T), *ZFP90* (rs1728785C>A), *ERCC2* (rs13181T>G), *PTPN23* (rs8180040T>A), and *CDH1* (rs9929218G>A, rs16260C>A) genes were significantly associated with reduced risk of colorectal adenomas, particularly in subjects with no family history of CRC. The most robust associations were observed for the rs10505477A>G and rs6983267G>T SNPs located in the long noncoding RNA (lncRNA) *CASC8* (cancer susceptibility candidate 8) gene. Of interest, both SNPs were firstly reported in 2007 as associated with CRC risk in two GWAS studies by Zanke *et al.* and Broderick *et al.*[9,10] Subsequent GWAS conducted in Asia and Europe corroborated the association between the rs10505477A and rs6983267G alleles and increased risk of CRC.[11,12] Allele frequencies of rs6983267 differ notably among ethnicities with values for the G variant ranging from 34% in Asians to 50% in Caucasians or nearly 100% in African Blacks. In our study, frequency of rs6983267 G allele was 57%, slightly higher than that reported in other European populations. *CASC8* rs6983267 and rs10505477 variants showed a very high linkage disequilibrium (LD) in our population (D' = 0.99, r^2 = 0.93) which agrees with data reported in HapMap for European populations (r^2 = 0.93). Unlike CRC, very few studies have addressed the contribution of *CASC8* rs6983267 and

Table 6. SNPs associated with risk of adenoma subtypes in FDR of patients with family history of CRC (cases)

			Adenoma		Low risk adenoma							High risk adenoma						
SNP	Genetic				No*	Yes**						No*	Yes**					
(Gen)	Model	Genotype	*p*-value[1]	FDR[2]	n	n	OR	95%	CI	*p*-value[1]	FDR[2]	n	n	OR	95%	CI	*p*-value[1]	FDR[2]
rs10795668	Cod	G/G	**0.006**	0.967	198	41	1.00			**0.003**	0.559	198	49	1.00			0.322	0.963
LINC00709		A/G			167	51	1.46	0.91	2.34			167	53	1.15	0.73	1.83		
		A/A			46	3	0.26	0.08	0.90			46	8	0.62	0.27	1.45		
	Dom	G/G	0.544	0.369	198	41	1.00			0.490	**0.013**	198	49	1.00			0.868	0.997
		A/G-A/A			213	54	1.18	0.74	1.87			213	61	1.04	0.67	1.62		
	Rec	G/G-A/G	**0.004**	0.197	365	92	1.00			**0.003**	**0.011**	365	102	1.00			0.169	0.884
		A/A			46	3	0.22	0.06	0.72			46	8	0.58	0.26	1.31		
rs11255841	Cod	T/T	**0.028**	0.967	211	43	1.00			**<0.001**	0.518	211	60	1.00			0.415	0.963
LINC00709		A/T			158	50	1.57	0.98	2.52			158	41	0.78	0.49	1.26		
		A/A			41	1	0.10	0.01	0.76			41	9	0.64	0.28	1.45		
	Dom	T/T	0.754	0.506	211	43	1.00			0.388	**0.002**	211	60	1.00			0.216	0.997
		A/T-A/A			199	51	1.23	0.77	1.95			199	50	0.75	0.48	1.18		
	Rec	T/T-A/T	**0.008**	0.197	369	93	1.00			**<0.001**	**0.002**	369	101	1.00			0.391	0.979
		A/A			41	1	0.08	0.01	0.61			41	9	0.71	0.33	1.57		
	Over	T/T-A/A	0.264	0.968	252	44	1.00			**0.008**	**0.033**	252	69	1.00			0.456	0.989
		A/T			158	50	1.87	1.17	2.99			158	41	0.84	0.53	1.33		
rs2228000	Cod	G/G	0.189	0.981	224	40	1.00			**0.030**	0.054	224	59	1.00			0.455	0.963
XPC		A/G			155	44	1.67	1.02	2.74			155	40	0.93	0.58	1.49		
		A/A			28	11	2.51	1.12	5.64			28	10	1.63	0.71	3.74		
	Dom	G/G	0.180	0.982	224	40	1.00			**0.013**	**0.047**	224	59	1.00			0.937	0.997
		A/G-A/A			183	55	1.80	1.13	2.88			183	50	1.02	0.65	1.59		
	Adi	GG,AG,AA	0.084	0.956	407	95	1.62	1.13	2.30	**0.008**	**0.033**	407	109	1.11	0.78	1.58	0.571	0.983
rs2427308	Dom	C/C	0.363	0.982	251	68	1.00			**0.038**	**0.033**	251	62	1.00			0.845	0.997
CABLES2		C/T–T/T			156	26	0.59	0.35	0.98			156	47	1.05	0.67	1.65		
	Over	C/C-T/T	0.234	0.972	267	75	1.00			**0.006**	**0.033**	267	66	1.00			0.722	0.989
		C/T			140	19	0.47	0.27	0.82			140	43	1.09	0.69	1.72		

OR: odds ratio. CI: confidence interval. FDR: False Discovery Rate. n: number of individuals. Rec: recessive genetic model. Dom: dominant genetic model. Cod: codominant genetic model. Adi: additive genetic model. Over: overdominant genetic model. *Number of patients without adenomas ** Number of patients with colorrectal adenomas.
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[2]p-values obtained after applying the False Discovery Rate (FDR) test for multiple corrections. FDR values <0.05 are highlighted in bold.

rs10505477 variants to colorectal adenomas risk. In line with our results, a GWAS study by Edwards *et al.* reported a protective effect of the rs10505477G allele against adenomas with OR values (OR: 0.87, additive model) similar to those observed for rs6983267T allele in our population (OR: 0.80, additive model).[32] In addition, a recent meta-analysis by Montzaneri *et al.* showed the association of the wild rs6983267G variant with increased risk of developing colorectal adenomas.[33] The molecular mechanisms by which *CASC8* rs6983267 and rs10505477 variants modify the risk of adenomas and / or CRC are still unknown. Some studies have speculated that SNPs in lncRNAs may influence gene expression through long range cis-regulatory elements.[34–36] *CASC8* rs6983267 and rs10505477 are located in the 8q24.21 chromosomal region, a desert region of coding genes bounded by the *FAM84B* and *MYC* genes. The proto-oncogen *MYC* is a target gene of the Wnt / β-catenin signaling pathway involved in early stages of colorectal carcinogenesis. It has been suggested that a DNA loop brings the rs6983267 genomic region close to the proto-oncogen MYC locus, and that this physical association may contribute to enhance MYC transcription.[34] Moreover, the SNP-enhancer region is transcribed into the recently described lncRNA *CCAT2* (colon cancer associated transcript 2) gene. It has been shown that rs6983267 allele G increases CCAT2 expression by interactions with transcriptional factors (TCF7L2) and subsequent up-regulation of *WNT* signaling target genes.[36]

Besides *CASC8* variants, the rs9929218G>A and rs16260C>A SNPs located in the *CDH1* gene were associated with a lower risk of colorectal adenomas in our study. The *CDH1* (cadherin 1) gene encodes a calcium-dependent glycoprotein (E-cadherin), member of the cadherin superfamily, which plays a key role in cell–cell adhesion mechanisms in epithelial tissues. Loss of function of *CDH1* gene *via* somatic mutation or promoter methylation has been shown to activate the wnt/β-catenin signal transduction pathway triggering tumor proliferation, invasion, and/or metastasis.[37] Houlston *et al.* first reported the association of the intronic rs9929218 A variant with lower risk of CCR.[13] Subsequently, Burnett-Hartmann *et al.* revealed the association of the wild rs9929218 G allele with increased risk of colorectal adenomas.[38] Concerning rs16260C>A, SNP in strong LD with rs9929218 (D' = 0.97; r^2 = 0.89) that lies within the *CDH1* promoter, a recent meta-analysis performed in European Caucasian populations described the association of the minor allele A with lower CRC risk.[39] In line with these findings we observed a protective effect of the rs16260 A variant against the development of colorectal adenomas. In this regard, presence of the wild rs16260 C variant has been related with promoter methylation of the *CDH1* gene and loss of function, finding which is biologically plausible with the protective effect of the opposite rs16260 A variant observed in our study. To our knowledge, this is the first research work reporting the link between rs16260 and risk of colorectal adenomas. Further studies with larger populations and different ethnic groups are required to conclusively assess the relevance of this SNP on the development of colorectal adenomas.

As previously mentioned, the intronic rs1728785C>A variant located in the *ZFP90* gene was associated with a lower risk of adenomas. The *ZFP90* (ZFP90 zinc finger protein) gen encodes a member of the zinc finger protein family that modulates gene expression. Barrett *et al.* first identified this variant among ulcerative colitis risk loci.[40] Moreover, a fine-mapping of CRC susceptibility loci at 8q23.3, 16q22.1 and 19q13.11 revealed the *ZFP90* rs1728785 SNP as the most likely target of the 16q22.1 genetic variation associated with increased CRC risk.[41] However, the functional relevance of rs1728785 on *ZFP90* expression or function remains unknown. It is plausible that this intronic polymorphism is in LD with other functional SNPs that may affect cancer risk. Interestingly, the *ZFP90* rs1728785 SNP was in high LD with the *CDH1* rs9929218 (D' = 0.77, r^2 = 0.49) and *CDH1* rs16260 (D' = 0.79, r^2 = 0.55) variants previously reported to be associated with lower risk of adenomas in our population. Both, *CDH1* and *ZFP90* genes are located at 16q22.1 chromosomal region. Functional studies have reported a significant relation between *CDH1* rs9929218 variants and the expression of *ZFP90*. In this regard, Carvajal-Carmona *et al.* observed that the rs9929218 minor allele A significantly regulated *ZFP90* expression by a cis-effect.[41] The scarcity of *ZFP90* association studies highlight the need to characterize the genetic variation defined by the rs1728785 SNP and the functional consequences affecting *ZFP90* expression or protein function.

Similar to *ZFP90*, there is very limited knowledge about the influence of *ERCC2* rs13181T>G and *PTPN23* rs8180040T>A gene polymorphisms on CRC and/or colorectal adenoma susceptibility. Concerning the later, Fernandez-Rozadilla *et al.* first reported the association of the rs8180040 variant and CRC in a GWAS performed in Spain.[19] According to the authors, the rs8180040 variant was inversely associated with CRC risk which is in agreement with the protective effect of the rs8180040 allele A against colorectal adenomas observed in our study.

Stratified SNP analysis by family history of CRC revealed some additional significant associations. Among them, the most remarkable findings were observed in the lnc-RNA *CASC8* and *LINC00709* (long intergenic nonprotein coding RNA 709) genes with the intronic *CASC8* rs10505477A>G, rs6983267G>T, and *LINC00709* rs10795668G>A, rs11255841 T>A variants being associated with reduced risk of adenomas in patients with no family history of CRC. Notably, no significant associations with risk of adenomas were observed in FDRs of patients with CRC (cases). A possible explanation for this finding could be the presence of rare high-penetrance mutations in genes yet to be discovered that may mask the effect of polymorphisms in low-penetrance genes associated with risk of adenomas in FDRs of patients with CRC. In agreement with our results, a Spanish case–control study

reported the association between the *CASC8* rs6983267 variant and adenoma risk.[42] Interestingly, subjects with family and/or personal history of CRC were excluded from our study, fact that corroborates the association observed in our study only in the subgroup of patients with no family history of CRC. The rs10795668G>A and rs11255841T>A variants are located in the *LINC00709* (long intergenic nonprotein coding RNA 709) gene which belongs, like *CASC8*, to the new category of lncRNAs with important regulatory functions in the expression of multiple genes. The rs1 0795668 SNP was firstly identified as a CRC risk factor by Tomlinson *et al.* in a European GWAS.[11] According to the authors, the rs10795668 variant was associated with a lower risk of CRC. However, subsequent studies performed in different populations showed less conclusive results.[24] In line with the findings reported by Tomlinson *et al.*,[11] the rs1 0795668 A variant showed in our study a protective effect against the development of colorectal adenomas in patients with no family history of CRC. The rs10795668 and rs11 255841 variants were in strong LD ($D' = 0.96$, $r^2 = 0.84$) in our population. Functionally, both SNPs are located near to the *DD431424* and *HV455515* genes, recently identified as important regulators of the hTERT region which has been reported to harbor several susceptibility loci for various types of cancers, including CRC.[43,44]

Taking together our results support the hypothesis that some SNPs previously identified as CRC susceptibility loci are also associated with early events in the adenoma-carcinoma colorectal sequence. Because subtypes of adenomas (LRAs/HRAs) show a different risk of developing advanced neoplasia we further analyzed the influence of genetic risk variants on the phenotypic expression of adenomas according to the family history of CRC.

Stratified analysis by type of adenoma (LRAs/HRAs) revealed the association of the *CASC8* rs10505477 and rs6983267 variants with reduced risk of HRAs, particularly in patients with no family history of CRC (controls). Of interest, *CASC8* rs10505477 and rs6983267 were also significantly associated with a reduced risk of LRAs in patients with no family history of CRC, being this protective effect stronger on the risk of LRAs than on the risk of HRA development. Our results are in the same direction as those reported by Zhang *et al.* among European Americans showing a stronger association of the wild *CASC8* rs6983267 G and rs10505477 A alleles with the development of advanced adenomas than with the development of nonadvanced adenomas.[45] In addition to *CASC8* variants, the rs10795668 and rs11255841 SNPs located in the lnc-RNA *LINC00709* gene were significantly associated with a lower risk of LRAs in both, cases and controls, suggesting their implication in early stages of CRC development regardless the presence of family history of CRC. In line with our findings, Burnett-Hartman *et al.*[38] reported a significant association between the more frequent rs10795668 G allele and increased risk of adenomas, being this association stronger on the risk of advanced adenomas compared to nonadvanced adenomas. Unlike the protective effect of the *CASC8* and *LINC00709* variants observed in our study, the intergenic variant rs4779584 (*GREM1- SCG5*) was significantly associated with an increased risk of HRAs, particularly in patients with no family history of CRC. Our results are in agreement with a recent case–control study by Zhang *et al.*[45] reporting the link between the rs4779584 T allele and increased risk of advanced adenomas and multiples adenomas. The rs4779584 variant is located in proximity to the *GREM1* (gremlin 1, DAN family BMP antagonist) gene which encodes the synthesis of a protein (Gremlin 1) that is involved in the signaling pathway mediated by TGF-β growth factor. This signaling pathway is mainly active in late stages of colorectal carcinogenesis which is in accordance with the association between rs4779584 and risk of HRAs observed in our study.[46,47] Our study showed two more genetic variants associated with risk of development LRA, rs2228000G>A in XPC gen and rs2427308C>T in CABLES2 gen, although the association was not so obvious and only in FDRs of patients with CRC. Thus, we found that HRA showed a stronger association with SNPs associated with CRC susceptibility than LRA suggesting that these SNPs may play a more important role in CRC promotion than in CRC initiation.

Finally, our study has several strengths and limitations. A comprehensive analysis of 99 SNPs previously reported to be associated with CRC risk, was carried out in a large homogeneous population of well-characterized Spanish Caucasian subjects (750 cases and 750 controls). To our knowledge, the current study is the first to show a significant effect of *CDH1* rs16260, *ZFP90* rs1728785, and *PTPN23* rs8180040 variants on colorectal adenoma susceptibility. Moreover, additional associations with specific histological subtypes were observed. The fact that these associations remained significant after False Discovery Rate multiple test, and in some cases Bonferroni correction, indicates that our results may not be a chance finding. However, some limitations should be also considered. In particular, and despite our study is one of the largest performed in Western populations, the sample size limited the power to detect small ORs. Taking into account the prevalence of the SNPs evaluated in our population and setting an α value of 0.05, the study had a power of 80% to detect ORs > 1.413 or < 0.727 except for the less prevalent variants (MAF: 0.02–0.10), with a power of 80% to observe ORs > 4.850 in the whole data set. As a result, it is possible that we could have missed minor statistical differences, especially when subgroup analyses were performed.

In summary, we have shown that some specific variants associated with CRC risk, namely rs10505477 and rs6983267 in the *CASC8* gene, and rs10795668, and rs11255841 in the lnc-RNA *LINC00709* gene, are also involved in the development of colorectal adenomas or specific adenomas subtypes. Moreover, we found that these associations were modified by the presence of family history of CRC. A deeper knowledge of

genetic factors related to colorectal adenoma risk can provide insight into the biological and genetic mechanisms relevant to initiation and progression of colorectal tumors. Our results may have significant implications for the identification of those patients at risk of CRC who would benefit from stricter cancer screening programs.

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Disclosures

The authors have no conflict of interest regarding this paper.

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