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Influence of Genetic Polymorphisms in Prostaglandin E2 Pathway (COX-2/HPGD/SLCO2A1/ABCC4) on the Risk for Colorectal Adenoma Development and Recurrence after Polypectomy

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OBJECTIVES: Deregulation of prostaglandin E_2 (PGE_2) levels reported in colorectal carcinogenesis contributes to key steps of cancer development. Our aim was to evaluate the influence of the genetic variability in *COX-2/HPGD/SLCO2A1/ABCC4* PGE_2 pathway genes on the development and recurrence of colorectal adenomas.

METHODS: A case-control study was conducted gathering 480 unscreened individuals and 195 patients with personal history of adenomas. A total of 43 tagSNPs were characterized using the Sequenom platform or real-time PCR.

RESULTS: Ten tagSNPs were identified as susceptibility biomarkers for the development of adenomas. The top three most meaningful tagSNPs include the rs689466 in *COX-2* (odds ratio (OR) = 3.23; 95% confidence interval (CI): 1.52–6.86), rs6439448 in *SLCO2A1* (OR = 0.38; 95% CI: 0.22–0.65) and rs1751051 in *ABCC4* genes (OR = 2.75; 95% CI: 1.58–4.80). The best four-locus gene–gene interaction model included the rs1346271, rs1863642 and rs12500316 single nucleotide polymorphisms in *HPGD* and rs1678405 in *ABCC4* genes and was associated with a 13-fold increased susceptibility (95% CI: 3.84–46.3, $P < 0.0001$, cross-validation (CV) accuracy: 0.78 and CV consistency: 8/10). Interesting, in low-risk patients the *ABCC4* rs9524821AA genotype was associated not only with a higher hazard ratio (HR = 2.93; 95% CI: 1.07–8.03), but half of these patients had adenoma recurrence at 60 months, considerably higher than the 21% noticed in low-risk patients.

CONCLUSIONS: Genetic polymorphisms in COX-2/PGE_2 pathway appear to contribute to the development of colorectal adenomas and influence the interval time to adenomas recurrence. The definition of risk models through the inclusion of genetic biomarkers might improve the adherence and optimization of current screening and surveillance guidelines for colorectal cancer prevention.

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INTRODUCTION

Colorectal adenomas are well-characterized colorectal cancer (CRC) precursors.[1] Although most adenomas are asymptomatic and do not progress into cancer, the majority of CRC will develop through the adenoma-cancer sequence on an average of 10–15 years.[2] Over one-third of people will develop at least one adenoma in their lifetime.[3]

CRC screening has been shown to reduce the incidence and CRC mortality through the endoscopic detection and removal of adenomas.[4,5] Still, these patients are at increased risk for developing metachronous adenomas or even cancer, with a recurrence rate of 40–50%.[6,7]

Deregulation of COX-2 expression, observed in half of adenomatous polyps, leads to an increased biosynthesis of prostaglandin E_2 (PGE_2).[8] The pleiotropic effects of higher levels of PGE_2 contribute to key steps of cancer development including stimulation of cell proliferation, angiogenesis, invasiveness and migration, inhibition of apoptosis and immunosurveillance.[9] The degradation of PG is mediated by the NAD^+-dependent 15-hydroxyprostaglandin dehydrogenase (15-PGDH), encoded by the *hydroxyprostaglandin dehydrogenase* (*HPGD*) gene, which directly counteracts the COX-2 oncogenic PGE_2 pathway.[10] Furthermore, low levels of rectal 15-PGDH were associated with increased adenoma recurrence.[11] The multidrug resistance-associated protein 4 (MRP4) and the prostaglandin transporter (PGT), encoded by the *ATP-binding cassete sub-family C member 4* (*ABCC4*) gene and *solute carrier organic anion transporter family, member 2A1* (*SLCO2A1*) genes, respectively, are the specific prostaglandin membrane transporters that regulate PGE_2 levels in the extracellular microenvironment.[12,13] PGT and

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MRP4 messenger RNA levels were reported to be inversely regulated in human CRC, with PGT expression being repressed and MRP4 up-regulated in CRC tissues and cell lines leading to higher levels of PGE_2 extracellularly thus exacerbating the effects of COX-2/PGE_2 pathway.[14]

Considering that the aforementioned genes are not only highly polymorphic but their expression span several folds, one could hypothesize that an unbalance in PGE_2 levels reflecting potential functional polymorphisms might influence colorectal carcinogenesis.

Our group has previously reported the involvement of several polymorphisms in *COX-2/HPGD/SLCO2A1/ABCC4* genes on CRC development.[15–17] Therefore, with this study we aimed to investigate whether tagSNPs in these four genes were also associated with earlier stages of colorectal tumor development. To the best of our knowledge this study is the first to evaluate the influence of polymorphisms in *HPGD*, *SLCO2A1* and *ABCC4* genes in the occurrence of colorectal metachronous lesions.

METHODS

Type of study and participants. A hospital-based case-control study followed by a retrospective cohort was designed involving a group of unscreened individuals and patients diagnosed with colorectal adenomas recruited at the Portuguese Oncology Institute of Porto (IPO-Porto) or Centro Hospitalar do Porto (CHP).

The Ethic committees at both institutes approved this project and a written informed consent was given before enrollment.

Group of controls. The method of sampling is displayed in Figure 1. Unscreened individuals between 50 and 75 years of age, with no clinical evidence of CRC or other oncologic malignancy were randomly recruited from the blood donor's service at IPO-Porto between July 2005 and February 2008.

Group of patients. Patients diagnosed with one or more adenomas between 1996 and 2008 were enrolled in this study after reviewing a colonoscopy database from the Gastroenterology departments at IPO-Porto and CHP. The inclusion criteria were as follows: (1) age between 50 and 75 years; (2) without history of inflammatory bowel disease or family history of colorectal tumors; (3) without previous diagnose of CRC; (4) with a total colonoscopy with good to excellent preparation at diagnosis; (5) a normal total colonoscopy with good to excellent preparation at least 1 year after the diagnosis, to exclude missed lesions at diagnosis, followed by (6) at least one total colonoscopy with good to excellent preparation with or without adenomas detection, to estimate the recurrence status.

Nearly three thousand individuals had history of adenomas, although only $<10\%$ complied with the inclusion criteria. Two hundred and fifty-six patients were included in this study. From these we were only able to obtain DNA samples from 195 patients. No differences were observed between demographic variables, lifestyle habits and tumor characteristics between these patients and the overall population of patients.

Collection and processing of biological samples. The DNA was extracted from formalin-fixed paraffin-embedded blocks of excised adenomas using the GRS Genomic DNA Kit—Tissue, following the manufacturer's protocol (GRiSP, Porto, Portugal). The use of formalin-fixed paraffin-embedded samples for single nucleotide polymorphism (SNP) genotyping was previously validated by comparing the genotypes from 20 DNAs isolated from fresh peripheral blood and paired formalin-fixed paraffin-embedded samples from CRC patients.[15]

Selection of polymorphisms. The strategy for polymorphisms selection and quality control has been described elsewhere.[15] In brief, 55 tagSNPs were included after being retrieved from a set of common SNPs in the Caucasian population of HapMap project (CEU): (1) with minor allele frequency equal or superior to 0.15; (2) within the coding region of the genes plus 2 Kb upstream and downstream; (3) with a r^2 superior to 0.8 and (4) that successfully converted to the Sequenom platform.

Statistical analysis. The Hardy–Weinberg equilibrium was tested by the Pearson's goodness-of-fit test to compare the observed vs. the expected distribution of genotypes among the control population.

Data analysis was performed using the computer software IBM Statistical Package for Social Sciences-SPSS (IBM Corp., Armonk, NY, USA) for Macintosh (version 19.0). Chi-square analysis was used to compare categorical variables, using a 5% level of significance. Non-parametric Mann–Whitney test was used to compare mean values between study groups. Odds ratio (OR) and its 95% confidence interval (CI) were calculated as a measure of the association between the genetic variants and the risk for the development of colorectal adenomas. The covariates age, sex, and smoking habits were included in the logistic regression analysis. A bootstrap resampling was used to assess the stability of risk estimates (1,000 replications). The false-positive report probability (FPRP) was used to confirm the noteworthiness of significant findings on the overall risk for colorectal adenoma development, according to the study by Wacholder *et al.*[18] This methodology helps answer the questions: "of all tested null hypothesis that were rejected, what is the percentage of false rejection?". Three factors determine the magnitude of FPRP: the prior probability of the hypothesis, i.e., the assumed probability of a true association between a genetic variant and a disease; α level or P value and; statistical power to detect the OR of the alternative hypothesis at a given α level or P value.[18]

The FPRP threshold was set at 0.5, recommended for underpowered initial studies[18] and a moderate-to-high prior probability range was assigned to detect an OR of 1.5 (0.01–0.10). This interval was assumed in view of the available epidemiological and functional data supporting the relevance of the genes here addressed in colorectal carcinogenesis.[8,11,14,15]

Haplotype analysis was performed at a gene level using the SNPStats software (www. http://bioinfo.iconcologia.net/SNPstats). The haplotype frequencies were estimated using the implementation of the EM algorithm coded into the *haplo.stats* package. The most frequent haplotype was automatically selected as the reference category. After excluding the genetic variations most likely to represent false-positive findings, all polymorphisms with significant associations were included within each gene.

The open-source multifactor dimensionality reduction software (version 3.0.2; www.epistasis.org) was used to assess

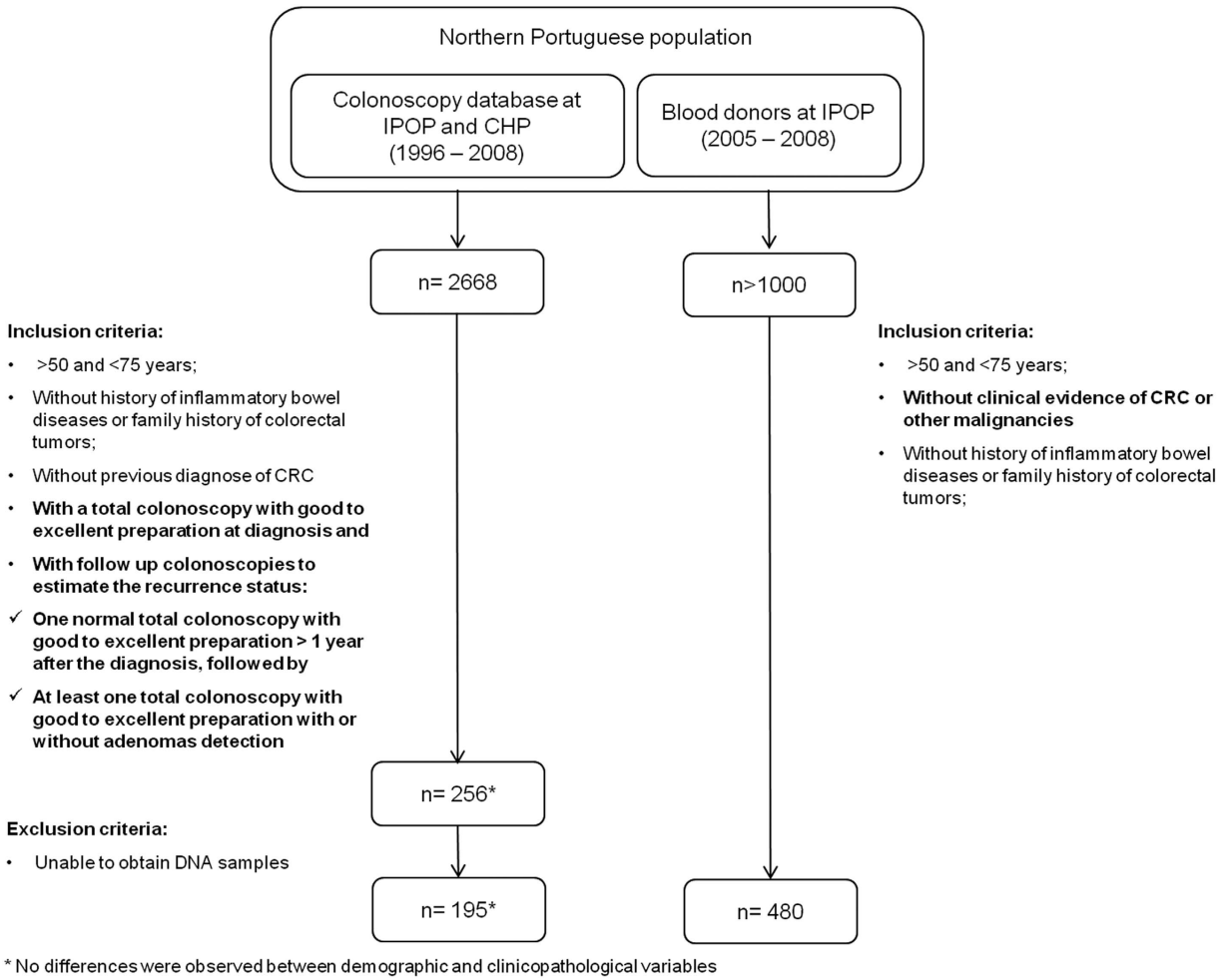

Figure 1 Methods of sampling for controls and cases.

potential gene–gene interactions between SNPs with statistical significant impact on colorectal adenoma genetic susceptibility. The fitness of an multifactor dimensionality reduction model was estimated by determining the testing accuracy and its cross-validation consistency. Using a 10-fold cross-validation method the data was divided into 10 sets, in which nine subsets were training sets and one subset was a test set. Hence, the cross-validation consistency is a measure of the number of times of 10 divisions of the data set the best model was extracted. Statistical significance was evaluated using a 1,000-fold permutation test to the compare observed testing accuracies with those expected under the null hypothesis of null association. Permutation testing corrects for multiple testing by repeating the entire analysis on 1,000 data sets consistent with the null hypothesis.

Kaplan–Meier curves were used to evaluate the correlation between the genetic variants and time to recurrence; log-rank statistical test was used for curves comparison.

RESULTS

Study population. A description of the population understudied is displayed in Table 1.

In over 70% of patients, less than three adenomas were detected. Most were located distally to the splenic flexure (82%) and were larger or equal to 10 mm in size (64%). Histologically, high-grade dysplasia was described in 33% of index adenomatous polyps.

High-risk patients for adenoma recurrence (adenoma with villous histology or high-grade dysplasia or $\geq$ 10 mm in size, or $\geq$ 3 adenomas) represented 72% of cases' population. The median follow-up time was 76 months (22–201) and metachronous lesions were identified in 46% of patients with personal history of adenomas. No differences were observed between these patients and the ones without adenoma recurrence during the follow-up period.

Risk estimates for the development of colorectal adenoma. A total of 43 SNPs were included in the risk estimate analysis. The description of selected SNPs and distribution of genotypes are displayed in Supplementary Tables 1 and 2, respectively. Twenty-three polymorphisms were associated with the development of colorectal adenomas, as observed in Table 2. To address for possible bias in these positive findings, the FPRP was also analyzed, with 10 polymorphisms

Table 1 Description of population

	Controls ($n=480$)	Adenomas ($n=195$)	*P* value	Recurrence		*P* value
				No	Yes	
Demographics						
Age (years)						
Mean (s.d.)	58 (4.90)	61 (6.78)	–	61 (6.64)	61 (6.74)	–
Median (min–max)	58 (50–69)	61 (50–75)	<0.001	61 (50–75)	60 (50–75)	0.698
Sex, n (%)						
Male	314 (65.4)	110 (57.3)	–	54 (49.5)	55 (50.5)	–
Female	166 (34.6)	82 (42.7)	0.159	48 (60.0)	32 (40.0)	0.154
Lifestyle behaviors						
Smoking status, n (%)						
Never-smokers	219 (60.3)	86 (71.7)	–	47 (54.7)	39 (45.3)	–
Ever-smokers	144 (39.7)	34 (28.3)	0.075	16 (47.1)	18 (52.9)	0.453
High-risk patients[a]						
No	–	53 (27.9)	–	27 (54.0)	23 (46.0)	–
Yes	–	137 (72.1)	–	74 (53.6)	64 (46.4)	0.963
Time of follow-up (mo)						
Mean (s.d.)	–	80.1 (39.5)	–	–	–	–
Median (min–max)	–	76 (22–201)	–	–	–	–
Polyps characteristics						
Number of adenomas						
Mean (s.d.)	–	2.15 (1.69)	–	1.98 (1.51)	2.38 (1.87)	–
Median (min–max)	–	1 (1–10)	–	1 (1–9)	2 (1–10)	0.119
<3	–	133 (70.7)	–	74 (55.6)	59 (44.4)	–
≥3	–	55 (29.3)	–	27 (49.1)	28 (50.9)	0.413
Tumor location, n (%)						
Distal	–	155 (82.0)	–	87 (56.1)	68 (43.9)	–
Proximal	–	34 (18.0)	–	15 (44.1)	19 (55.9)	0.203
Size, n (%)						
<10	–	67 (35.6)	–	37 (55.2)	30 (44.8)	–
≥10	–	121 (64.4)	–	65 (53.7)	56 (46.3)	0.843
Morphology, n (%)						
Pedunculated	–	76 (46.1)	–	42 (55.3)	34 (44.7)	–
Sessile	–	89 (53.9)	–	42 (47.2)	47 (52.8)	0.301
Histological Grade, n (%)						
Low-grade dysplasia	–	127 (67.2)	–	67 (52.8)	60 (47.2)	–
High-grade dysplasia	–	62 (32.8)	–	35 (56.5)	27 (43.5)	0.632
Histological type, n (%)						
Tubular	–	30 (46.1)	–	16 (53.3)	14 (46.7)	–
Tubulovillous	–	20 (30.8)	–	14 (70.0)	6 (30.0)	–
Villous	–	15 (23.1)	–	9 (60.0)	6 (40.0)	0.446
Metachronous adenomas						
No	–	102 (54)	–	–	–	–
Yes	–	87 (46)	–	–	–	–

max, maximum; min, minimum; mo, months.
[a]risk stratification for adenoma recurrence based on the endoscopic findings at baseline colonoscopy. Low-risk patients: 1–2 tubular adenomas <10 mm in size with low -grade dysplasia; High-risk patients: patients with adenomas with villous histology or high-grade dysplasia or ≥10 mm in size, or ≥3 adenomas.

retaining their association with colorectal adenoma onset. Different prior probabilities within the 0.01–0.1 range were assumed, with the highest cut-off of 0.1 assigned to the rs689466 and rs2555639 polymorphisms in *COX-2* and *HPGD* genes, respectively, considering the predicted biological impact and previous epidemiological evidence in colorectal carcinogenesis.[15,17,19,20] FPRP values below 0.5 suggest that the observed associations could represent false-positive reports.

The most noteworthy genetic variants include the rs689466 in *COX-2* (OR = 3.23; 95% CI: 1.52–6.86, $P=0.002$), rs2555639 and rs12500316 in *HPGD (*OR = 2.48; 95% CI: 1.36–4.53, $P=0.003$ and OR = 0.49; 95% CI: 0.31–0.78, $P=0.002$, respectively), rs6439448 in *SLCO2A1* (OR = 0.38; 95% CI: 0.22–0.65, $P<0.001$) and rs9524821, rs1751051 and rs1678405 in *ABCC4* genes (OR = 2.38; 95% CI: 1.39–4.09, $P=0.002$; OR = 2.75; 95% CI: 1.58–4.80, $P<0.001$ and OR = 0.41; 95% CI: 0.27–0.63, $P<0.001$, correspondingly), with an individual associated OR ≥ 2 for the development of colorectal adenomatous polyps, or corresponding OR ≤ 0.5.

The involvement of genetic variants in PGE_2 pathway genes on the recurrence of adenomas was negligible upon the FPRP analysis, potentially indicating false-positive associations (FPRP > 0.5; see Supplementary Table 3) or low power, considering we had <200 patients.

Haplotype analysis for the development of colorectal adenomas. The frequencies of derived haplotypes from *HPGD*, *SLCO2A1,* and *ABCC4* genes are presented in Table 3. In *ABCC4* gene, the most frequent haplotype, the GTT, was present in 27% of controls and used as the reference one. The block carrying the rs9524821A and rs1751051A alleles boosted even further the susceptibility

Table 2 Risk estimates for the involvement of *COX-2/HPGD/SLCO2A1/ABCC4* polymorphisms in colorectal adenoma development (only statistical significant data is presented)

SNP	Model of inheritance	aOR	95% CI	*P* value	$P_{bootstrap}$	FPRP prior probability		
						0.01	0.05	0.1
COX-2								
rs689466	Recessive (AA/AGvsGG)	3.23	1.52–6.86	0.002	0.001	0.908	0.654	**0.472**[a]
HPGD								
rs2555639	Recessive (TT/TCvsCC)	2.48	1.36–4.53	0.003	0.002	0.859	0.538	**0.356**[a]
rs2612656	Dominant (AAvsAG/GG)	0.47	0.23–0.94	0.033	0.035	0.953	0.794	0.646
	Recessive (AA/AGvsGG)	3.20	1.22–8.41	0.018	0.009	0.967	0.848	0.726
rs8752	Recessive (AA/AGvsGG)	1.94	1.09–3.44	0.023	0.036	0.924	0.701	0.526
rs1346271	Dominant (GGvsGC/CC)	0.55	0.35–0.85	0.008	0.013	0.785	**0.411**	**0.249**
rs1863642	Dominant (GGvsGT/TT)	0.55	0.36–0.85	0.007	0.008	0.785	**0.411**	**0.249**
rs12500316	Dominant (CCvsCT/TT)	0.49	0.31–0.78	0.002	0.002	0.729	**0.340**	**0.196**
SLCO2A1								
rs4241362	Recessive (TT/TCvsCC)	3.90	1.80–8.43	0.001	0.001	0.876	0.575	**0.391**
rs6439448	Dominant (CCvsCA/AA)	0.38	0.22–0.65	<0.001	0.002	0.670	**0.280**	**0.156**
rs9821091	Dominant (GGvsGA/AA)	0.62	0.40–0.96	0.033	0.044	0.895	0.621	**0.437**
	Recessive (GG/GAvsAA)	1.77	1.02–3.06	0.041	0.048	0.936	0.738	0.571
rs4241365	Recessive (TT/TCvsCC)	2.60	1.30–5.22	0.007	0.006	0.921	0.692	0.516
SLCO2A1								
rs7625035	Recessive (AA/AGvsGG)	2.68	1.71–6.11	0.020	0.026	0.957	0.812	0.672
rs1131598	Dominant (AAvsAG/GG)	0.58	0.41–0.84	0.018	0.052	0.629	**0.245**	**0.133**
rs10935090	Recessive (CC/CTvsTT)	5.18	1.33–20.17	0.018	0.002	0.979	0.901	0.812
ABCC4								
rs9524821	Recessive (GG/GAvsAA)	2.38	1.39–4.09	0.002	0.003	0.780	**0.405**	**0.244**
rs869951	Dominant (GGvsGC/CC)	0.60	0.39–0.92	0.018	0.019	0.858	0.537	**0.354**
rs1751051	Recessive (TT/TAvsAA)	2.75	1.58–4.80	<0.001	0.001	0.691	**0.300**	**0.169**
rs2892713	Recessive (CC/CTvsTT)	2.50	1.12–5.58	0.025	0.006	0.959	0.819	0.682
rs4612933	Recessive (CC/CTvsTT)	3.03	1.35–6.79	0.007	0.005	0.941	0.754	0.593
rs4148476	Recessive (TT/TGvsGG)	3.22	1.41–7.36	0.005	0.005	0.940	0.751	0.588
rs1678405	Dominant (TTvsTC/CC)	0.41	0.27–0.63	<0.001	0.001	**0.261**	**0.064**	**0.031**
	Recessive (TT/TCvsCC)	0.15	0.04–0.63	0.010	0.010	0.979	0.897	0.805
rs1751031	Recessive (AA/AGvsGG)	2.99	1.11–8.00	0.030	0.016	0.971	0.867	0.756
rs7993878	Recessive (GG/GAvsAA)	3.14	1.09–9.01	0.033	0.024	0.975	0.882	0.780

aOR, Odds ratio, logistic regression (Forward:conditional model) including age, sex and smoking habits as covariates; CI, confidence interval; FPRP, false-positive report probability; SNP, single nucleotide polymorphism.
[a]A prior probability of 0.1 was assumed for the rs689466 and rs2555639 considering the available epidemiologic and functional data;[15,17,19,20] For all other polymorphisms without previous evidences, a lower prior probability of 0.05 was considered. Only statistical significant associations are presented ($P<0.05$). Bold for FPRP <0.5, i.e., the statistical associations are less likely to represent false-positive findings.

Table 3 Haplotype frequencies between patients and controls and risk estimates for their involvement in adenoma development

Gene/ haplotype	Cases (%)	Controls (%)	aOR	95% CI	*P* value
HPGD[a]					
C-G-G-T	35.0	28.0	1	Reference	–
C-G-G-C	18.6	10.9	1.04	0.64–1.70	0.87
C-C-G-T	15.3	9.1	1.11	0.68–1.82	0.67
T-G-G-T	1.1	12.6	0.05	0.01–0.15	**<0.001**
C-C-T-T	0	10.4	–	–	–
C-C-T-C	0.8	8.8	0.06	0.01–0.33	**0.001**
SLCO2A1[b]					
A-C	71.6	60.8	1	Reference	–
G-C	17.2	20.8	0.68	0.49–0.95	**0.024**
A-A	8.4	15.4	0.50	0.33–0.76	**0.001**
ABCC4[c]					
G-T-T	19.8	26.9	1	Reference	–
A-T-T	26.8	19.7	1.85	1.19–2.86	**0.006**
G-C-T	15.3	17.0	1.18	0.71–1.98	0.52
G-T-A	14.3	14.4	1.51	0.89–2.56	0.13
A-T-A	17.3	3.9	3.90	2.28–6.65	**<0.001**
G-C-A	5.7	6.1	0.99	0.46–2.12	0.98

aOR, Odds ratio adjusted for age, sex and smoking habits; CI, confidence interval; OR, Odds ratio.
[a]SNPs order: rs12500316-rs1346271-rsrs1863642-rs2555639.
[b]SNPs order: rs1131598-rs6439448.
[c]SNPs order: rs9524821-rs1678405-rs1751051. Bold for $P<0.001$.

for colorectal precancerous lesion reported in the individual analysis (OR = 3.90; 95% CI: 2.28–6.65, $P<0.001$).

Gene–gene interaction analysis in the development of colorectal adenoma. To address possible interactions between the noteworthy SNPs from the main analysis, an exhaustive multifactor dimensionality reduction approach was employed and Table 4 summarizes the best interactive models obtained. The best four-locus model achieved the highest testing accuracy of 78% for predicting the development of colorectal adenomas, with a cross-validation consistency of 8/10. This interaction model included the rs1346271, rs1863642, and rs12500316 polymorphisms in *HPGD* gene and rs1678405 in *ABCC4* gene and was associated with a 13-fold increased risk for the development of adenomas (95% CI: 3.84–46.3, $P<0.0001$).

Influence on the time to adenoma recurrence and crude risk. We next inquired if polymorphisms in these key genes in PGE_2 pathway could influence not only the time but also the crude risk for adenomas recurrence at 36, 60, and 120 months, following the recommendations for post-polypectomy colonoscopy surveillance (Table 5).[21] Although no difference was observed on the time to adenoma

Table 4 MDR analysis for the colorectal adenoma risk prediction

Best model	CV accuracy	CV consistency	aOR	95% CI	*P* value
rs1346271, rs12500316	0.6964	9/10	5.41	1.88–15.5	0.001
rs1346271, rs1863642, rs12500316	0.7006	6/10	5.51	1.90–15.9	0.001
rs1346271, rs1863642, rs12500316, rs1678405	0.7816	8/10	13.3	3.84–46.3	<0.001

aOR, Odds ratio adjusted for age, sex and smoking habits; CI, confidence interval; CV, cross-validation; MDR, multifactor dimensionality reduction.

Table 5 Influence of genetic variations in *COX-2/HPGD/SLCO2A1/ABCC4* on the time to recurrence of colorectal adenomas and crude risk of recurrence at 36, 60, and 120 months of follow-up

		Recurrence (%)	aOR (95% CI)	aHR (95% CI)	Time to recurrence[a] (min–max)	Crude risk for recurrence (%)		
						36 mo	60 mo	120 mo
Low-risk individuals								
Global	–	46	–	–	112 (100–124)	2	21	86
SLCO2A1								
rs9820625	AA/AC	32	10.71 (1.17–98.24)	3.33 (1.22–9.10)	115 (100–130)	3	16	70
	CC	80	–	–	85 (62–108)	0	29	100
ABCC4								
rs9524821	GG/GA	41	–	2.93 (1.07–8.03)[a]	122 (97–147)	4	16	78
	AA	43	–	–	107 (57–157)	0	48	100
rs1678396	TT	50	–	0.20 (0.07–0.60)	94 (90–98)	0	18	100
	TC/CC	39	–	–	122 (95–149)	3	18	80
rs2274403	AA	50	–	0.26 (0.08–0.83)	85 (29–140)	0	44	69
	AG/GG	39	–	–	122 (109–135)	3	23	83
rs3742106	AA	21	5.36 (1.25–23.04)	5.78 (1.61–20.8)	135 (77–193)	0	8	72
	AC/CC	59			105 (84–126)	3	27	92
rs6492763	TT	73	0.18 (0.04–0.74)	0.26 (0.07–0.91)	93 (55–130)	6	38	100
	TC/CC	15	–	–	176 (–)	0	7	66
rs869951	GG/GC	35	–	–	114 (105–123)	3	17	89
	CC	75	–	–	66 (48–84)	0	50	100
High-risk individuals								
Global		46	–	–	105 (87–123)	14	27	82
SLCO2A1								
rs1131598	AA/AG	44	–	3.23 (1.49–7.02)	105 (86–124)	13	23	81
	GG	73	–	–	67 (59–74)	19	59	100
rs7616492	GG	57	–	–	94 (78–110)	14	40	94
	GA/AA	39	–	–	121 (98–143)	13	20	74
rs7340717	GG/GT	44	–	–	115 (88–142)	12	26	81
	TT	57	–	–	94 (43–145)	26	47	93
ABCC4								
rs1678405	TT	39	2.09[a] (1.04–4.23)	1.75[a] (1.05–2.91)	109 (89–129)	7	22	77
	TC/CC	57	–	–	90 (76–104)	23	35	88

aOR, Odds ratio: logistic regression with center as covariate; aHazard Ratio, cox regression including center as covariate; CI, confidence interval; max, maximum; min, minimum; mo, month.
[a]$P>0.05$ upon the bootstrap analysis based in 1,000 samples.

recurrence (112 vs. 105 months, $P=0.788$) or recurrence rate (46%, $P=0.996$) between the high and low-risk patients, 14% of all adenomatous polyps recurred at 36 in the high-risk group in contrast to the 2% reported in low-risk patients. In addition, nearly 95% (18/19) of metachronous advanced adenomas were described in the high-risk group, with 28 and 67% being diagnosed at 36 and 60 months (data not shown).

The rs9524821AA genotype not only was associated with a nearly threefold increased susceptibility in the cox regression analysis (95% CI:1.07–8.03, $P=0.036$), but half of patients carrying this genotype had adenoma recurrence at 60 months, considerably higher than the 21% noticed in low-risk patients. Similarly, patients' carriers of rs2274403AA genotype had a lower interval until recurrence (85 (29–140) vs. 122 (109–135), $P=0.011$) with 44% of metachronous tumors developing by 36 months (vs. 23% for AG/GG).

DISCUSSION

CRC remains a major clinical and public health challenge that could be averted by applying the current knowledge about CRC prevention and improving the adherence to established screening guidelines.[4,5]

The search for susceptibility biomarkers in colorectal carcinogenesis might reveal an important tool to select unscreened individuals to CRC screening or even to complementary chemopreventive strategies with nonsteroidal anti-inflammatory drugs (NSAIDs) by allowing the identification of individuals at higher risk for the development of colorectal tumors.

The efflux-dominated flow of PG during carcinogenesis as a reflection of an increased expression of COX-2 and MRP4, and down regulation of 15-PGDH and PGT lead to an accumulation of PGE_2 in the extracellular milieu culminating

in the activation of a plethora of pathways that stimulate tumor development.[14]

In the present study, we addressed the role of 43 tagSNPS in four candidate genes (*COX-2/HPGD/SLCO2A1/ABCC4*) of COX-2/PGE_2 pathway on the development and recurrence of colorectal adenomatous polyps in a northern Portuguese population. Recently, using the same tagSNPs approach and targeting the same pathway we also identified the rs689466A>G polymorphism in *COX-2*, the rs1346271G>C in *HPGD*, the rs6439448C>A in *SLCO2A1* and the rs1751051T>A in *ABCC4* genes polymorphisms as susceptibility biomarkers for CRC, supporting the associations reported here and the role they might portray in colorectal carcinogenesis.[15]

The homozygous GG genotype for the rs689466 SNP, also known as -1195A>G *COX-2* polymorphism, associated presently with a threefold higher predisposition, was previously related with a higher risk for duodenal adenomatosis in patients with familial adenomatous polyposis (FAP).[22] Although representing a hereditary syndrome, deregulation of COX-2 expression was observed in normal and duodenal adenomas of FAP patients.[23] Furthermore, our group, in an earlier study observed a higher transcriptional activity in HCT116 and HCA-7 CRC cell lines transfected with *COX-2* promoters' encompassing the rs689466G allele.[19] Thereby, providing a biological plausibility for the epidemiological observations.

Thompson *et al.*[20] first associated the rs2555639T>C SNP located at 17.74 kb upstream the 5′UTR of *HPGD* gene with a 40% increased risk for CRC in TT homozygous carriers. Surprisingly, in our population, this SNP not only appears to be more relevant in early stages of colorectal carcinogenesis, but the opposing rs2555639CC genotype was linked to colorectal adenomas onset. This conflicting data might reflect population stratification involving different genetic ancestry, considering that the initial study involved participants from the Kentucky Surveillance, Epidemiology and End Results (SEER) registry most likely with northern or western European ancestry (English, German and Irish ancestry). Furthermore, the rs12500316C>T tagSNP in *HPGD* gene also displayed a protective role in colorectal adenoma onset in a previous study reported by Edwards *et al.*[24]

The PGT and MRP4 specific PG membrane transporters are encoded by highly polymorphic genes. Still, the study of genetic variants in *SLCO2A1* and *ABCC4* genes on the etiology of malignant diseases has been rather neglected.[15,25] In our population, A allele carriers of the rs6439448 tagSNP in *SLCO2A1* presented a 60% protection for colonic adenoma development. Biologically, the rs6439448 SNP tags two other polymorphisms with predicted impact on PGT expression: the rs2370512T>A in the 3′UTR could affect the binding of microRNAs and stability of messenger RNA and the non-synonymous rs34550074G>A SNP at codon 396 codes for two different amino acids (Ala396Thr) with potential repercussion on protein structure and function (SNPinfo software).

Remarkably, homozygous mutations in *HPGD* and more recently in *SLCO2A1* gene were identified as causative agents for the development of primary hypertrophic osteoarthropathy (PHO).[26,27] Similarly to neoplastic tumor genesis, increased levels of PGE_2 play a role in the pathogenesis of PHO, thus reinforcing the impact that genetic variability in these genes might portray in disease development by disrupting the normal 15-PGDH and PGT levels or activity.[27]

More interesting, individuals carrying the haplotype containing the A allele for the rs9524821 and rs1751051 SNPs in *ABCC4* gene had a nearly fourfold increased susceptibility. The *in silico* analysis did not provide any biological clue for the involvement of these polymorphisms in MRP4 expression or function.

The common disease-common variant (CD-CV) hypothesis predicts that complex polygenic diseases develop from the additive or multiplicative effect of low penetrance genes.[28] Here, a 13-fold increased predisposition was noticed in the multi-locus analysis, supporting the role that common variants portray in colorectal carcinogenesis.

The current post-polypectomy guidelines recommend endoscopic surveillance based on risk stratification upon the endoscopic findings at baseline colonoscopy.[21] In the present study, we observed that polymorphisms in the COX-2/PGE2 pathway, particularly on the *ABCC4* gene, appear to influence not only the hazard ratio for the development of metachronous adenomas, but perhaps more importantly, the probability of recurrence considering the surveillance intervals currently recommended. As an example, the individuals carrying the rs9524821AA genotype in the low-risk group presented a nearly threefold increased hazard ratio for adenoma recurrence and nearly half of them developed metachronous lesions by 60 months (vs. 16%, for G allele carriers).

Further research with larger and independent populations should be warranted as the data here presented, although suggesting the involvement of genetic variants in PGE_2 pathway in the development and recurrence of adenomas, derives from an underpowered proof-of-concept study.

Furthermore, and following a retrospective study design we cannot rule out recall bias that could decrease the availability and accuracy of collected data, compromising our ability to estimate possible gene-environment interactions or selection bias. First, our control population was represented by unscreened individuals. Although if this was the case, stronger associations would be expected; and second, an enrichment towards the diagnosis of advanced adenomas was observed (72%), albeit this study included all patients with personal history of adenomas that gathered the inclusion criteria. This might potentially be explained since cases were recruited from tertiary care hospitals, including a cancer specialized center. In fact, patients from the cancer institute had a higher frequency of high-grade dysplasia (45 vs. 19%) and sessile adenomas (65 vs. 40%). When studying the recurrence of adenomatous polyps the recruitment center was included as a covariate in the logistic regression analysis. Moreover, no difference in the distribution of genotypes was observed considering histopathological features.

In the absence of a replication study, the bootstrap resampling or cross-validation consistency methods were used to test the robustness, performance and internal validity of our analyses.

Functional studies are also needed to evaluate the repercussion of the aforementioned SNPs on protein expression/function to allow a deeper understanding of their real contribution on cancer development.

In this study, we observed the involvement of several polymorphisms in *COX-2/HPGD/SLCO2A1/ABCC4* genes in colorectal adenoma development and recurrence. If corroborated by future research, the definition of genetic profiles might

have the potential to represent a tool to optimize current screening and surveillance guidelines.

CONFLICT OF INTEREST

Guarantor of the article: Carina Pereira, PhD.
Specific author contributions: Planning and conducting the study, collecting and interpreting data, and drafting the manuscript: Carina Pereira; collecting and interpreting data, and drafting the manuscript: Sara Queirós; collecting data: Ana Galaghar; interpreting data: Hugo Sousa; planning and collecting data: Ricardo Marcos-Pinto, Pedro Pimentel-Nunes and Catarina Brandão; planning and interpreting data, and drafting the manuscript: Rui Medeiros; planning and conducting the study, interpreting data and drafting the manuscript: Mário Dinis-Ribeiro; all authors have approved the final draft submitted.
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Potential competing interests: None.

Study Highlights

WHAT IS CURRENT KNOWLEDGE

- Deregulation of COX-2/PGE2 pathway is observed in colorectal cancer and is involved in key steps of tumor development.
- The genetic background plays a role in cancer development.

WHAT IS NEW HERE

- Genetic variability in COX-2/HPGD/*SLCO2A1/ABCC4* genes influences the risk for the development and recurrence of colorectal adenomas.
- Polymorphisms in *SLCO2A1* and *ABCC4* alter the interval time to adenoma recurrence and crude risk at 36, 60, and 120 months.

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