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The risk for colorectal cancer and polymorphisms of APC gene

Oana Andrada Alexiu[1,✉], Mihai Toma[1],
Irina Radu[1], Anne-Marie Crăciun[2] and Dănuț Cimponeriu[1]

SUMMARY. Colorectal cancer (CRC) is a complex genetic disease which appears as a result of interactions between multiple genetic and non-genetic factors. In this study we have analyzed the association between four polymorphisms of APC gene (*rs41116, rs465899, rs2229992* and *rs2019720*) and colorectal cancer in Romanian population. After receiving the informed consent, blood samples were obtained from individuals with CRC (M:F=95:85) and from healthy persons (M:F=27:33). Genomic DNA was extracted from peripheral blood samples and the APC gene polymorphisms were assessed by PCR-RFLP. The promoter polymorphism rs2019720 was associated with CRC. Thus, the CC genotype (OR 2.307) and allele C (OR 1.843) increased the disease risk, while the AA genotype (OR 0.453) and allele A (OR 0.543) decreased the CRC risk. We found that the APC polymorphism *rs2019720* was associated with CRC in Romanian population.

Keywords: Adenomatous polyposis coli, colorectal cancer, polymorphisms

Introduction

The mutations in adenomatous polyposis coli (*APC*) gene are involved in the development of colorectal cancer (CRC) (Fearnhead *et al.,* 2001; Fodde, 2002). Germline mutations were linked to familial adenomatous polyposis (FAP), while somatic mutations occurs in approximately 80% of sporadic cases (Laurent-Puig *et al.,* 1998; Goss and Groden, 2002). Most frequently patients are carriers of frameshift mutations (68%) which are associated with synthesys of proteins with abnormal structure (e.g. truncated proteins) and functions. For example, the missense germline mutations, p.I1307K and p.E1317Q, were frequently identitifed in sampels from CRC patients (Frayling *et al*., 1998). Also, codons 1286 and 1450 represents hotspots for somatic mutations which underlying the occurrence of premature stop codons (Fodde, 2002; Laurent-Puig *et al.,* 1998).

[1] Departament of Genetics, University of Bucharest, Human Genome, Bucharest, Romania.
[2] Nicolae Paulescu NIDNMD, Bucharest, Romania.
✉ ***Corresponding author: Oana Andrada Alexiu****, Intrarea Portocalelor street, no. 1-3, Bucharest, E-mail: alexiu.andrada@gmail.com*

In addition to these mutations the role of single nucleotide polymorphisms (SNPs) of APC in genetic predisposition to CRC is a subjects of discussions (Almeida *et al.,* 1996; Tranah *et al.*, 2005). The variants of these SNP have a low penetrance and present significant interpopulational differences. As a consequence, the disease risk may not be identical in different ethnic groups (Chen *et al*., 2006). However, for several SNPs the association with CRC was reconfirmed in several independent studies (e.g. g.1458T>C, g.4479G>A and g.5268T>G, p.D1822V) (Slattery *et al*., 2001; Tranah *et al.*, 2005; Chen *et al*., 2006).

Until now no large-scale screening for APC gene variants in Romanian population has been conducted. In this study we have analyzed the relationship between four polymorphisms of APC gene (*rs41116, rs465899, rs2229992* and *rs2019720*) and CRC in Romanian population.

Materials and methods

Blood samples were obtained from 180 individuals with CRC and from 60 healthy subjects after receiving the informed consent (Table 1). Genomic DNA was extracted from peripheral blood samples and the APC gene polymorphisms were assessed by PCR-RFLP using already published protocols (Sieber *et al*., 2002; Cui *et al*., 2005). Briefly, each PCR reaction (10 μL) contains about 50 ng genomic DNA, 1×PCR buffer, 1.5 mmol/L $MgCl_2$, 1 unit Taq DNA polymerase, 100 μmol/L dNTP and 0.5 μmol/L of each primer. The annealing temperature for each polymorphism was 55°C. The amplicons were digested with SspI (*rs41116*), MspI (*rs465899*) and RsaI (*rs2229992* and *rs2019720*) restriction enzymes.

Statistical analysis was performed using *StatsDirect* (version 2.8.0) software. Chi-square test (χ 2) was used to compare the distribution of genotypes and alleles in patients and control groups. A p value < 0.05 was considered statistically significant.

Table 1.

Characteristics of analyed subjects

	CRC patients	**CRC controls**
Median age	62.5	58.5
Interval	51-79	46-67
Men / Women	95 / 85	27 / 33

Results and discussion

The distribution of APC genotype are presented in Table 2. All investigated polymorphisms were distributed in accordance with the Hardy-Weinberg equilibrium.

Table 2.

The distribution of APC gene polymorphism in patients and control groups

	Genotypes	**CRC patients**	**CRC controls**	**OR (95%CI)**	**χ^2 (p)**
rs2019720	CC	66	14	**2.30** (1.17-4.53)	6.05 (***0.01***)
	CA	73	31	0.78 (0.43-1.42)	0.63 (0.42)
	AA	21	15	**0.45** (0.21-0.95)	4.49 (***0.03***)
	HWE (p)	0.9	0.79		
rs2229992	CC	38	11	1.38 (0.65-2.93)	0.74 (0.38)
	CT	85	37	0.70 (0.38-1.29)	1.28 (0.25)
	TT	37	12	1.2 (0.57-2.5)	0.24 (0.61)
	HWE (p)	0.42	0.07		
rs465899	GG	29	12	0.77 (0.36-1.65)	0.42 (0.51)
	GA	85	35	0.80 (0.44-1.47)	0.47 (0.48)
	AA	49	13	1.59 (0.79-3.21)	1.73 (0.18)
	HWE (p)	0.28	0.19		
rs41116	TT	49	14	1.45 (0.73-2.88)	1.13 (0.28)
	TC	83	32	0.94 (0.52-1.7)	0.03 (0.84)
	CC	28	14	0.69 (0.33-1.43)	0.96 (0.32)
	HWE (p)	0.48	0.6		

We observed that rs2019720 polymorphism is associated with CRC. Thus, CC genotype (OR 2.3) and C allele (OR 1.84) increased the disease risk, while the AA genotype (OR 0.45) and the A allele (OR 0.54) seems to be protective factors for CRC.

These results are in concordance with the data obtained by Huang and contributors that reported a significant difference between cases and controls only for rs2019720 polymorphism (Huang *et al*., 2012).

The rs2019720 polymorphism is located in the promoter region of the APC gene and is expected to be functional and to be a real marker for CRC predisposition. However, the haplotype analysis may describe much better the disease risk. Huang and collaborators found a strong LD between rs2019720 and rs6594646 but demonstrated that no haplotype is significant associated with CRC (Huang *et al*., 2012).

We previously tested by PCR multiplex the presence of mutations in APC gene in samples collected from 16 patients (10 men and 6 women) and 21 first and second degree relatives and found no mutation in the codon 1061 of APC gene (Toma *et al*., 2008). The data obtained during the present study complete the data regarding predisposition for CRC in Romanian population.

Conclusions

In this study the rs2019720 is associated with susceptibility to CRC in Romanian population.

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