P53 AND DCC POLYMORPHISMS AND THE RISK FOR COLORECTAL CANCER IN ROMANIAN PATIENTS – A PRELIMINARY STUDY

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Abstract. Inactivation of tumor suppressor genes *p53* and *DCC* has been frequently observed in colorectal cancer. The aim of this case-control study was to test possible association between polymorphisms *g.32008376A>G* (*rs714*) of *DCC* gene and g.7175464A>G (*rs1625895*) of *p53* gene and colorectal cancer risk in Romanian patients. We investigate these two polymorphisms by PCR-RFLP in individuals with colorectal cancer (n=120, M:W=74:46) and healthy persons (n=60, M:W=32:28). We observed that GG genotype of both genes confer protection for CRC (OR_{DCC} 0.34, 95%CI 0.18-0.66, OR_{p53} 0.28, 95%CI 0.14-0.55). The presence of *DCC* AA (OR 2.97, 95%CI 0.97-9.08) and *p53* GA (OR 3.86, 95%CI 1.89-7.87) genotypes are associated with an increased risk for CRC. The alleles A of both markers are associated with the risk for disease (OR 2.87, 95%CI 1.49-5.50, respectively 3.54, 95%CI 1.81-6.91). We also observed that coinheritance of *DCC* GG genotype and *p53* GG (OR 0.36) or *p53* GA (OR 0.23) confer protection for CRC. These apparent discordant results obtained for the *p53* gene may be the result of interaction with other markers or a selection bias. Our findings indicate that the *p53* and *DCC* polymorphisms are associated with a risk of CRC in Romanian patients.

Keywords: colorectal cancer, *p53* gene, *DCC* gene, genetic polymorphism, PCR-RFLP.

INTRODUCTION

Colorectal tumorigenesis represents a multiple-step process [3, 19]. This requires mutational activation of oncogenes and the loss of several tumor suppressor genes [11, 12]. The genetic model of sporadic colorectal cancer (CRC) progression is based on sequence adenoma - carcinoma [8]. This model was proposed over 20 years ago. Since then, different genes mutations and new pathways implicated in CRC were identified [9, 25].

The inactivation of tumor suppressor genes by deletion and mutation of the remaining allele is considered to play an important role in carcinogenesis [5]. The loss of heterozygosity (LOH) and the loss of expression of the tumor suppressor genes *p53* and *DCC* gene have been frequently found in the CRC [2, 14].

The *DCC* gene (18q21.3) encodes a netrin-1 receptor component with functions in cell migration, cell cycle arrest and apoptosis. LOH of the *DCC* gene is a late event associated with poor differentiation, malignant progression and poor prognostic for CRC patients [22].

The *p53* tumor suppressor gene (17p13.1) encodes a multifunctional transcription factor that is involved in transcription, DNA replication and repair, differentiation, development and programmed cell death [1, 13]. Alterations of *p53* gene are associated with more advanced stages of transition from benign to malignant growth, the loss of the *p53* gene being a marker for the conversion of adenoma to carcinoma [20, 24].

Recent reports show that single nucleotide polymorphisms (SNPs) of these two genes confers predisposition for CRC [6, 7, 15, 23, 26]. The aim of this study was to test possible association between polymorphisms *g.32008376A>G* (*rs714*) of *DCC* gene and g.7175464A>G (*rs1625895*) of *p53* gene and CRC risk in Romanian patients.

MATERIALS AND METHODS

Subjects. Between January 2008 and June 2009, blood samples and medical information regarding cancer type, tumour location and clinical evolution from 120 patients (M:W=74:46) diagnosed with CRC were obtained at Cantacuzino Hospital (Bucharest). Sixty healthy controls (M:W=32:28), without known family history of malignancies and cardio-vascular diseases were selected from persons who attended N. Paulescu Institute (Bucharest) for routine analysis. The Research Ethics Committee of N. Paulescu Institute approved this study and the research is in concordance with principles of the Declaration of Helsinki. After informed consent was obtained from each participant, three ml of blood were collected in a tube containing EDTA.

Genotyping. DNA was extracted from peripheral blood leukocytes using a commercial extraction kit (Genomic Wizard DNA Purification Kit, *Promega*). These two polymorphisms were detected by PCR-RFLP. Briefly, 60 ng DNA were amplified in a final volume of 10 µL, containing 1×PCR buffer, 1.5 mmol/L $MgCl_2$, 100 µmol/L dNTP, 0.5 µmol/L of each primer (Table 1) and 1 unit Taq DNA polymerase (*Promega*). The PCR was performed in a *Corbett research thermocycler*. The annealing temperature for each reaction is given in Table 1. For both polymorphisms, amplicons (5 µl) were digested with 4 U of MspI (*Promega*) at 37 °C, for three hours. The DNA fragments were separated by electrophoresis on 2% agarose gels for *DCC* and on 8% PAGE for *p53*. The gels were visualized using *Bio-Imaging System* after ethidium bromide staining. Dimension of amplicons and restriction fragments are presented in Table1.

Statistical analysis. Distribution of alleles and genotypes among cases and control subjects were counted and compared with *Hardy–Weinberg* equilibrium. Chi-square test (χ^2) was used to compare

the distribution of genotypes and alleles in patients and control groups. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using the relevant 2 × 2 contingency table. A p value < 0.05 was considered statistically significant. Statistical analysis was performed using SISA programs [21].

Table 1. Primer sets used in PCR-RFLP.

Gene	Ancestral Allele	Amplicon size (bp)	Primers	Annealing temperature (°C)	References
DCC	G	Allele A 396 bp	*5'-TGCACCATGCTGAAGATTGT-3'*	55	[17]
		Allele G 256 + 140 bp	*5'-AGTACAACACAAGGTATGTG-3'*		
P53	A	Allele A 107 bp	5'-AGGTCTGGTTTGCAACTGGG-3'	62	[16, 18]
		Allele G 63 + 44 bp	5'-GAGGTCAAATAAGCAGCAGG-3'		

RESULTS

The *p53* and *DCC* polymorphisms were genotyped in 120 patients with CRC and 60 healthy controls. The male proportion was 61.6% in cases and 53.3% respectively in controls. The mean age was 63.7±4.8 in cases and 62.3±3.8 in controls. The colorectal tumours were localized in colon and sigmoid (62.3% of cases) and in rectum (37.7% of cases).

The frequencies of genotypes and alleles of these two polymorphisms are shown in Table 2. The genotypes of these two SNPs were distributed in accordance to *Hardy-Weinberg* equilibrium expectation in cancer and control groups.

We observed that GG genotype of both genes confer protection for CRC (OR 0.34, 95%CI 0.18-0.66, respectively 0.28, 95%CI 0.14-0.55). The presence of *DCC* AA (OR 2.97, 95%CI 0.97-9.08) and *p53* GA (OR 3.86, 95%CI 1.89-7.87) genotypes are associated with an increased risk for CRC. The alleles A of both markers are associated with the risk for disease (OR 2.87, 95%CI 1.49-5.50, respectively 3.54, 95%CI 1.81-6.91).

Table 2. The distribution of *DCC* and *p53* genotypes and alleles between cancer and control group.

Distribution / Genes	Genotype / alleles	Cancer N (%)	Control N(%)	OR (95%CI) [a]	χ^2(p) [b]
DCC	**GG**	31 (25.8)	30 (50)	**0.34** (0.18-0.66)	10.42 (*0.0012*)
	GA	68 (56.7)	26 (43.3)	1.71 (0.91-3.19)	2.85 (0.0913)
	AA	21 (17.5)	4 (6.7)	**2.97** (0.97-9.08)	3.92 (*0.0475*)
	G	130 (54.2)	86 (71.7)	**0.31** (0.1-0.94)	4.59 (*0.0321*)
	A	110 (45.8)	34 (28.3)	**2.87** (1.49-5.50)	10.42 (*0.0012*)
p53	**GG**	50 (41.7)	43 (71.6)	**0.28** (0.14-0.55)	14.41 (*0.0001*)
	GA	62 (51.7)	13 (21.7)	**3.86** (1.89-7.87)	14.811(*0.0001*)
	AA	8 (6.6)	4 (6.7)	1 (0.28-3.46)	0.1 (0.7512)
	G	162 (67.5)	99 (82.5)	1 (0.28-3.46)	0.1 (0.7512)
	A	78 (32.5)	21 (17.5)	**3.54** (1.81-6.91)	14.416 (*0.0001*)

Observation: [a] odds ratio and 95% confidence interval; [b] Values of Chi squares and p.

Table 3. The combined genotypes of *p53* and *DCC* polymorphisms

Genotypes	P53 GG	P53 GA	P53 AA
DCC GG	12 / 20	17 / 8	2 / 2
DCC GA	31 / 21	35 / 4	2 / 1
DCC AA	7 / 2	10 / 1	4 / 1

Observation: (N patients / N controls)

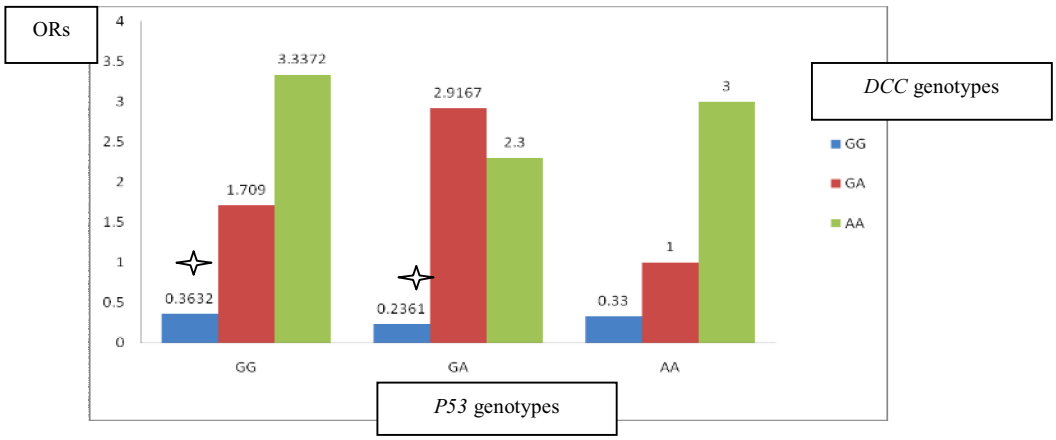

Figure 1. Graphical representation of ORs for combined genotypes of *p53* and *DCC* polymorphisms.
Observation: ✦ $p < 0.05$

The distribution of combined genotypes of these two polymorphisms is shown in Table 3. We observed that the coinheritance of *DCC* GG genotype and *p53* GG (OR 0.36) or *p53* GA (OR 0.23) confer protection for CRC (Fig. 1).

DISCUSSIONS

This case-control study was conducted in order to elucidate the possible association between polymorphisms of *p53* and *DCC* genes and CRC development in Romanian subjects.

Until now, it have been described some polymorphisms of *DCC* gene that were associated with CRC. Thus, polymorphism at codon 201 of the *DCC* gene was associated with type of carcinoma, stage of disease and prognosis of CRC [26]. For another SNP (rs2298606) the CC genotype was associated with the tumors located to the left colon [23]. For polymorphism *rs714* we did not find any article referring to CRC. We have found that GG genotype and G allele are associated with protection for CRC; meanwhile, AA genotype and A allele are associated with risk for CRC.

Among SNPs of *p53* gene, the polymorphism at codon 72 has been widely studied being associated with CRC susceptibility and disease outcome [6, 7, 15]. The *rs1625895* was reported to be associated with a high degree of endometrial cancer [4] and lung cancer risk [27], but not associated with breast cancer [10] and CRC [15]. We find that GG genotype of this polymorphism confers protection; meanwhile, GA genotype and A allele are associated with risk for CRC.

The *p53* GA genotype is more frequent whereas the GG genotype is less frequent in cancer lot than in controls. These results may be explained by the selection bias or by the fact that G allele may predispose to an increasing rate of mutation's accumulation, a rapid evolution of malignancies and with apparition of other malignant diseases. The GA genotype may have an opposite effect and may be more common between patients with a single malignant disease.

This is the first report that evaluates the combined effects of these two polymorphisms and CRC risk. We found two combined genotypes *DCC* GG / *p53* GG and *DCC* GG / *p53* GA that confer protection for CRC. However the degree of protection attributed to these combined genotypes (OR=0.36, respectively OR=0.23) is similar with the value obtained for DCC marker. The effect of *p53* marker seems to be discordant in this study. Thus, it seems to be protective in association with *DCC* GG, although in univariate analysis *p53* GA genotype confers risk for disease. These apparent discordant data may be the result of interaction with other markers from p53 gene [28].

In conclusion, our findings indicate that the *p53* and *DCC* polymorphisms are associated with a risk of CRC in Romanian subjects.

Acknowledgements. This work was supported by the Romanian Ministry of Education and Research (Research project CNCSIS TD No 51/2008). We are grateful to patients for their collaboration and to the staff of Cantacuzino Hospital and N Paulescu Institute for their cooperation.

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