

No Association with Risk for Colorectal Cancer of the Insertion/Deletion Polymorphism which Affects Levels of Angiotensin-converting Enzyme

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Abstract. *Background:* In the light of the established association of angiotensin-converting enzyme (ACE) with several types of cancer, the possible contribution of the insertion/deletion (I/D) polymorphism that affects ACE gene expression, in the development of colorectal cancer was investigated. *Materials and Methods:* DNA samples of 92 patients with colorectal cancer (adenocarcinomas) and 102 healthy controls were examined by allele-specific polymerase chain reaction followed by electrophoretic analysis. The resulting allele and genotype frequencies of the patients were compared to those of the controls by Fischer's exact test and odds ratios. *Results:* No statistical differences were observed between healthy controls and patients with colorectal cancer regarding either genotype distribution or low expression I allele frequency. *Conclusion:* The ACE I/D polymorphism is not a genetic predisposing factor concerning the risk for colorectal cancer.

Colorectal carcinoma (CRC) accounts for 9.4% of total cancer cases, while it is the third and fourth most frequent carcinoma in women and men, respectively (1). Its pathogenesis involves alterations in oncogenes and tumor suppressor genes, as well as other factors, such as poor hygiene, drugs, diet or smoking (2-4). Hereditary syndromes with autosomal dominant inheritance, such as familial adenomatous polyposis, account for only about 2-6% of CRC cases, indicating that the majority of these malignancies are not inherited in a clear Mendelian

fashion (4). Therefore, genetic association studies are necessary for the identification of any contribution conferred by subtle DNA alterations. Recently, such association studies have pinpointed functional DNA polymorphisms in angiogenesis, inflammation and thrombosis-related genes with increased risk for several carcinomas (5-23).

A factor related to both with thrombosis and cancer is angiotensin-converting enzyme (ACE), a major participant in the rennin-angiotensin system that converts angiotensin I to the vasoconstrictor angiotensin II (24). ACE has been implicated in the pathology of several carcinomas such as breast, prostate, endometrial and oral cancer, since its inhibition suppresses tumor growth and angiogenesis (25-30).

ACE activity is determined by the well-characterized insertion/deletion (I/D) polymorphism (31). This genetic polymorphism involves either the absence (D allele) or the presence (I allele) of a 287 bp Alu repeat sequence inside intron 16 of the ACE gene (32). Homozygotes for the I allele display half of the plasma ACE level compared to the homozygotes for the D allele, whereas the ID heterozygotes display an intermediate level (32). In order to determine whether the ACE I/D polymorphism is associated with increased risk for colorectal cancer this polymorphism was investigated in patients with colorectal adenocarcinoma and healthy controls.

Materials and Methods

The subjects included 92 patients surgically treated for colorectal cancer (adenocarcinomas) within the last 5 years and 102 healthy blood donors of Greek origin. DNA was extracted from biopsies of patients and from blood samples of controls. Each biopsy was characterized pathologically as well as in regard to Dukes' cancer stage. Molecular detection was performed by allele-specific polymerase chain reaction amplification and gel electrophoretic analysis, as described previously (33). The frequencies of alleles

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Table I. Prevalence of ACE I/D polymorphism in healthy controls, the whole group of patients and patients with colorectal cancer at cancer stages A,B and C,D (according to Dukes' stage).

Genotypes	Controls		Patients		Patients with cancer stages A,B			Patients with cancer stages C,D		
	N(%)	N(%)	Fisher's p-value	OR (CI)	N(%)	Fisher's p-value	OR (CI)	N(%)	Fisher's p-value	OR (CI)
Mutant I/I	6 5.9%	15 16.3%	<i>p</i> =0.09	3.12 (0.79-12.24)	7 15.6%	<i>p</i> =0.21	3.19 (0.70-14.50)	8 17.02%	<i>p</i> =0.13	5.56 (1.05-29.52)
Normal D/D	52 51%	50 54.4%		1 (referent)	25 55.6%		1 (referent)	25 53.19%		1 (referent)
Carrier D/I	44 43.1%	27 29.4%	<i>p</i> =0.16	0.69 (0.33-1.45)	13 28.9%	<i>p</i> =0.27	0.78 (0.32-1.92)	14 29.8%	<i>p</i> =0.34	0.70 (0.29-1.68)
Total	102 100%	92 100%			45 100%			47 100%		
Prevalence of I allele										
I allele frequency	27.5%	31%	<i>p</i> =0.50		30%	<i>p</i> =0.67		30%	<i>p</i> =0.49	
Carrier frequency of I allele	49%	45.7%	<i>p</i> =0.67		44.4%	<i>p</i> =0.72		46.81%	<i>p</i> =0.86	

OR: Odds ratio; CI: 95% confidence interval.

and genotypes in patients were compared with those of the control group and bilateral statistical analysis was performed using Fischer's exact test with the level of significance set at *p*<0.05. Age-adjusted odds ratios were calculated using the Maentel-Haenzel method with 95% confidence interval (CI), while the age criterion for the adjustment of odds ratios was set at 55 years.

Results

Table I shows ACE genotypes and I allele frequency observed in healthy controls, the whole group of patients and patients with colorectal cancer at Dukes' stages A,B (early) and C,D (advanced). In the control group, the genotypes were in Hardy-Weinberg equilibrium. The genotypes in the patient group did not reveal any significantly statistical difference in comparison to healthy controls. Additionally, there was no significant difference in the overall I allele and carrier frequencies of patients in comparison to healthy controls. Finally, there was no statistical difference in the frequencies of the two ACE alleles due to categorization of gender, age or cancer stages (early versus advanced ones).

Discussion

ACE has been associated with several cancer types including breast, prostate, endometrial and oral carcinomas by influencing tumor cell proliferation, tumor cell migration and angiogenesis (25-28, 33). The data obtained in the present study revealed no differences between healthy controls and

patients with colorectal cancer in regard to either genotype distribution or low expression I allele frequency.

Some studies investigating the ACE I/D polymorphism in various carcinomas have revealed an association of the high expression D allele with risk for carcinogenesis (25-28). These studies investigated sex hormone-related neoplasias and suggested that the increased ACE expression affected oncogenesis mainly by facilitating angiogenesis, due to the higher production of angiotensin II. In contrast, in a recent study investigating the role of ACE in oral cancer it was suggested that oral oncogenesis was driven through a bradykinin-related pathway and not through angiotensin II (33). Accordingly, and in the light of our results, oncogenesis in the digestive system probably does not involve angiotensin II.

Further genetic association studies of additional risk factors for colorectal cancer should be effected for better elucidation of the underlying pathogenic mechanisms and with a view to better prevention measures in certain individuals.

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