Protective Effect of *Cox-2* Allelic Variants on Risk of Colorectal Adenoma Development in African Americans

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Abstract. *Background: Recent evidence indicates that single nucleotide polymorphisms (SNPs) in the Cox-2 gene may modulate the risk of colorectal adenoma development. Patients and Methods: We explored possible associations between Cox-2 polymorphisms and risk of adenoma development in an African American case–control study comprising 72 cases of advanced adenomas and 146 polyp-free controls. An exhaustive approach of genotyping 13 haplotype-tagging SNPs (ht SNPs) distributed over the entire COX-2 gene was used. Results: Statistically significant inverse associations were observed between the heterozygous genotypes at the 5229 G>T polymorphism in intron 5 [odds ratio (OR)=0.42; confidence interval (CI)=0.19-0.92; p=0.03] and at the 10935 A>G polymorphism in the 3' flanking region downstream from the poly A signals (OR=0.39; CI=0.18-0.83; p=0.01) and the risk for colorectal adenoma development. Conclusion: The data from our pilot study suggest that allelic variants of the COX-2 gene significantly influence the risk of adenoma development in the African American population.*

Colon cancer accounts for approximately 10% of all cancer-related deaths and remains the third deadliest killer among cancer types in the United States (1). Epidemiological data show that African Americans have higher age-specific incidence and mortality rates and lower 5-year survival rates compared to Caucasians (2). Although reasons for this disparity are not clear, evidence implicates genetic, environmental and lifestyle factors as contributors to this multi-factorial disease (3).

There is mounting evidence that chronic inflammation is involved in the etiology of cancer. Previous studies have reported an association between genetic variants of pro-inflammatory genes and the risk of developing colorectal adenoma and carcinoma (4-6). One such gene, encoding the enzyme cyclooxygenase-2 (Cox-2) plays a significant role in inflammation and carcinogenesis (7). Epidemiological observations as well as randomized prevention clinical trials have provided evidence for a significant role of Cox-2 in colon carcinogenesis (8-11). More recently, several studies have explored association between genetic variants of *Cox-2*, alone or in interaction with environmental factors, and risk of developing colorectal adenoma/carcinoma mostly in Caucasians (12-15).

Relatively few studies have addressed the influence of genetic variants of *Cox-2* on cancer risk in the African American population. A polymorphism in African Americans replacing the amino acid valine with alanine at position 511 in exon 10 of *Cox-2* has been described to reduce the risk of colorectal cancer (16, 17). Another study reported different patterns of association between the genetic variants in the regulatory regions of *Cox-2* and prostate cancer risk in three different ethnic populations including African Americans (18). This observation is not surprising as patterns of genetic polymorphisms may vary within and between populations. The haplotype block structures of human genome containing regions of high linkage disequilibrium are of shorter size and reduced diversity in African Americans compared to Caucasians (19). To better understand the significance of genetic variations in the *Cox-2* gene in influencing colorectal cancer risk in African Americans, we used a case–control study of advanced adenomas to exhaustively analyze a possible

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Table I. *Polymorphisms, primers and probes used in this study.*

Polymorphism	Forward primers	Reverse primers	Reporter _VIC	Reporter _FAM
466 (A>C)	AGAAAGGCTTCC	CACCAGGTACCTCA	TCTCATGAA	TCATGCAG
	TAGATGAGATGGA	ATTTGTAGAAGT	GAATCAG	AATCAG
663 (GT>del)	AGGACTTAGGACATA	GGAGCATGTGAGG	CACTTTTCTGGT	CTTTTCTGGT
	ACTGAATTTTCTATTTT	GTGAGATACT	GTGTGTATA	GTGTGTGTATA
861 (G>A)	GCACTACCCATGA	TTCAGTTGCCTGG	ACGAGAATAAA	CGAGAATAG
	TAGATGTTAAACAA	GCTTATTG	AAATTAGCC	AAAATTAGC
2331 (C>T)	GTGACTTGGGA	GGCTCATAATGAT	CACGGAGTTCT	ACGGAGTTC
	AAGAGCTTGGA	CAGTGCTTGTG	TTCGGACT	TTTCGAACT
5072 (A>C)	CAGGTATTGTTATTT	CGGCATAATCATG	TTAGTACTGCA	AGTACTGCAA
	GTAATTTGACCCTTGT	GTACAATGTGTT	AAATGTTATG	ACTGTTATG
5229 (G>T)	TGGATTTCAATAG	TGTTTAACGGAATTAAT	CTTTTTTAGAATTACC	CTTTTTTATAATTACCAT
	CATAGCTTCAAGTT	ATACTATATTGAGCTTA	ATATCATCATAGT	ATCATCATAGTGAA
5625 (G>A)	AATGAAATATCAGGT	CAGTTAAAAAGTTAAGG	ACTTAGTTATTAC	CTTAGTTATTA
	ATGCTTCCTTTGACT	AACACATTTTTAGGGA	CACTTATAC	CCGCTTATAC
6064 (T>C)	GTTTTGAGTAAATGAC	TCAAAAGATAGCTATTT	ACTCACACACT	ACTCACACAT
	AAGATGTGGTAAATGA	TATCAGTCATGCTTACA	CTATATAC	TCTATATAC
8344 (TTATA>del)	AAATGAGTTTTGAC	CCATCTTGTGACAGTG	TTCAACTTATAAG	TTCAACTTATATTATA
	GTCTTTTTACTTGA	TTTAAGTATTCA	AACGAAAGTAA	AGAACGAAAGTA
8494 (C>T)	TCCATGATGCATTAGA	GCACTGATACCTGT	CTTTTGGTC	ACTTTTGGT
	AGTAACTAATGTTTGA	TTTTGTTTGATGA	ATTTTTC	TATTTTTC
10494 (T>C)	TCTGCTGACAAA	CTTATCTTTTACATAAGTTA	CACTGAAACAT	ACTGAAACAT
	ACCTGGGAATTT	AATACACATTTGTCTGAGG	TCGCATACA	TCACATACA
10848 (G>A)	ACTGTGTTGGAAAA	TCTTCTAGACTAGGC	CTTTACAGAAG	CTTTACAGAAGA
	TGTCTAGTTTGTGTA	AATGAAAATAAGCT	ATGAMAAACA	TGGMAAAC
10935 (A>G)	AAGAAGAAGAAAAAAAT	GCCCAACTTTGTATA	ACTTTGTGC	CTTTGTGC
	ACACAATAAGGCAAAGA	ATTTCCTCCTCTT	CTCCTTCA	CCCCTTCA

Numbers for polymorphism refer to positions in the Genbank entry AY382629 and as detailed at http://pga.gs.washington.edu/data/ptgs2/ptgs2.ColorFasta.html

association between *Cox-2* polymorphisms and colorectal cancer by genotyping 13 haplotype-tagging single nucleotide polymorphisms (htSNPs) in the *Cox-2* gene.

Materials and Methods

Patient selection. The study was approved by the Howard University Institutional Review Board. Study participants were recruited from patients referred for colonoscopy to the gastroenterology division at Howard University Hospital between September 2000 and October 2003. Indications for colonoscopy included rectal bleeding, irregular bowel habit, weight loss, family history of colon polyp/cancer, personal history of colon polyp and routine screening. Cases were eligible if colonoscopy resulted in a first diagnosis of colorectal adenomatous or hyperplastic polyp as confirmed by histology. Patients with a history of inflammatory bowel disease, malabsorption, any cancer, current or past chemotherapy or interferon treatment were excluded. Patients with distal or proximal polyps and with adenomatous or hyperplastic pathology, as determined by independent pathologists were selected as cases. Based on these criteria, 72 patients qualified as cases. Controls had to be free of all polyps and self-described with no previous history of colorectal adenomas/cancer. All patients were as African Americans. Clinical and demographic data collected on each patient included race, gender, past medical history, family history of colorectal polyp/cancer and information on smoking, alcohol consumption and medication use. DNA was extracted from samples from 72 patients and 146 controls.

Genotyping. The htSNPs of the *Cox-2* gene for the population of African American descent, together with the respective primers and probes used in this study are displayed in Table I. The positions of the polymorphisms refer to the Genbank entry AY382629 and as detailed at http://pga.gs.washington.edu/data/ptgs2/. All assays were designed and developed using Assay-by-Design (Applied Biosystems Inc, CA, USA). All oligo primers and probes were synthesized by Applied Biosystems, Inc. Assays were validated and optimized using in-house collected human DNA samples. Positive control DNAs of known genotypes as well as a no-template control were run on each assay plate for quality control. All SNPs were tested by the Taqman assay using the MGB chemistry (Applied Biosystems, Inc.) and the ABI 7900HT Sequence Detector. SDS 2.1 (Applied Biosystems, Inc.) was used to determine the genotype calls. Specific experimental details about genotyping will be provided upon request from the authors.

Data analysis. Odds ratios (ORs) were estimated using logistic regression models with the PROC LOGISTIC function of the SAS software package (version 9.1; SAS Institute, Cary, NC, USA) adjusting for gender and smoking. Departure from Hardy–Weinberg equilibrium was assessed by comparing the expected to observed genotype frequencies using the asymptomatic Pearson's χ^2 test.

Table II. *Characteristics of cases and controls.*

Characteristic	Cases	Controls	OR	95% CI	*P*-value
Gender					
Male	48	85	1	-	-
Female	25	67	0.66	0.37-1.18	0.21
Age (years)					
<60	35	88	1	-	-
>60	37	64	1.45	0.83-2.55	0.25
Body mass index					
<25	11	32	1		
25-30	20	40	1.45	0.60-3.47	0.39
>30	22	61	1.04	0.45-2.43	0.91
Smoking status					
Non-smoker	33	90	1	-	-
Current	15	15	2.73	1.20-6.19	**0.03**
Former	25	45	1.52	0.81-2.84	0.25
Alcohol					
Never	29	84	1	-	-
Current	33	69	0.63	0.28-1.41	0.26
Former	13	24	0.88	0.40-1.95	0.75
Aspirin					
Never	22	124	1	-	-
Yes	11	27	0.81	0.38-1.75	0.75

OR, odds ratio; CI, confidence interval.

Results

Characteristics of the study population and the association with colorectal cancer in this group of cases and controls are displayed in Table II. The only highly significant positive association was observed between current smokers and the risk of adenoma development [odds ratio (OR)=2.73, 95% confidence interval (CI) =1.20-6.19, p=0.03). A non-significant association was also present between former smokers and adenoma development (OR=1.52, CI=0.81-2.84, p=0.25).

The data of the association analysis for the main effect of the 13 polymorphisms distributed over the entire *Cox-2* gene are displayed in Table III. Of the 13 htSNPs, an intronic polymorphism and another in the 3' flanking region (FR), when adjusted for gender and smoking, were associated with a lower risk of adenomas. Adjusting for age and smoking also resulted in very similar associations (data not shown). Individuals with the heterozygous genotype at the intron 5-5229 had a statistically significant decrease in the risk of developing adenomas (OR=0.42, CI=0.19-0.92, p=0.03). Similarly, a highly significant protective effect for adenoma risk was observed in individuals with the heterozygous genotype at position 3'FR-10935 (OR=0.39, CI=0.18-0.83, p=0.01). The risk of adenoma in individuals with the variant homozygous genotypes at intron 5-5229 and 3' FR-10935, however, was no different from that of the control group.

Table III. *COX-2 genotypes and the risk of advanced colorectal adenoma.*

Genotype	Cases/controls	OR	95% CI	*P*-value
466 rs689462				
AA	45/79	1.00	-	-
AC	20/40	1.00	0.51-1.93	0.99
CC	4/14	0.61	0.18-2.03	0.42
663 rs689464				
GT	39/72	1.00	-	-
GT/del	26/45	1.09	0.58-2.05	0.80
del	2/14	0.29	0.06-1.38	0.12
861 rs20415				
GG	60/111	1.00	-	-
AG	9/21	0.80	0.34-1.88	0.61
AA	3/17	0.29	0.08-1.04	0.06
2331 rs2745557				
CC	51/93	1.00	-	-
CT	18/31	1.10	0.55-2.20	0.78
TT	0/18	-	-	-
5072 rs4648274				
AA	53/97	1.00	-	-
AC	15/29	0.99	0.48-2.05	0.98
CC	1/5	0.38	0.04-3.34	0.38
5229 rs20432				
TT	16/21	1.00	-	-
TG	32/90	0.42	0.19-0.92	**0.03**
GG	25/34	1.05	0.44-2.50	0.91
5625 rs2066826				
GG	31/56	1.00	-	-
AG	27/57	0.82	0.43-1.57	0.61
AA	12/16	1.38	0.57-3.36	0.49
6064 rs4648276				
TT	55/94	1.00	-	-
CT	14/32	0.70	0.34-1.45	0.34
CC	1/4	0.42	0.04-4.09	0.45
8344 rs4648291				
TTATA	26/54	1.00	-	-
TTATA/del	29/54	1.06	0.54-2.07	0.86
del	15/23	1.54	0.68-3.56	0.31
8494 rs5275				
CC	23/52	1.00	-	-
CT	31/58	1.20	0.61-2.34	0.60
TT	16/26	1.53	0.68-3.45	0.30
10494 rs689470				
TT	12/18	1.00	-	-
CT	26/61	0.54	0.22-1.32	0.18
CC	32/53	0.86	0.36-2.06	0.74
10848 rs4648306				
GG	55/95	1.00	-	-
AG	14/30	0.77	0.37-1.61	0.49
AA	1/5	0.32	0.04-2.94	0.31
10935 rs4648308				
GG	23/38	1.00	-	-
AG	22/66	0.39	0.18-0.83	0.01
GG	24/38	0.85	0.39-1.84	0.68

Ancestral alleles are treated as wild-type. OR, odds ratio; CI, confidence interval. Values are adjusted for gender and smoking.

Besides the polymorphisms at intron 5- 5229 and 3'FR-10935, two other polymorphisms in the promoter region showed a trend for a protective effect for adenoma development. There was a marginally significant lower risk of adenoma development in individuals with the rare homozygous genotype at the –861 position (OR=0.29, CI=0.08-1.04, p=0.06) and a statistically non-significant protective trend for the risk of adenomas (OR=0.29, CI=0.06-1.38, p=0.12) in individuals with another rare homozygous variant at the –663 polymorphism (Table III).

Discussion

To our knowledge, our pilot study represents the first exhaustive approach to determine the influence of genetic variants of *Cox-2* on the risk of colorectal adenoma development in African Americans. We evaluated 13 htSNPs with a minor allele frequency ranging between 0.13-0.43 that were distributed over the entire *Cox-2* gene and captured most common variations in the African American population. Two polymorphisms located in intron 5 and in the 3' FR showed a protective effect for adenoma development.

A reduced risk of adenoma development in African Americans in carriers of the heterozygous genotype at intron 5-5229 in the *Cox-2* gene is consistent with the previous finding of the protective effect of this polymorphism on development of colorectal adenoma in Caucasians (12). Interestingly, a Swedish study reported a protective effect of the same polymorphism (rs20432) (referred to as position +3100) for prostate cancer (20). It is also noteworthy that, similar to our study in African Americans, the heterozygous but not the variant homozygous genotype at intron 5-5229 had an inverse association with prostate cancer risk in a Swedish population (20). Intronic sequences are believed to harbor transcriptional regulatory elements. The intronic variants may therefore modulate disease risk by regulating gene expression, gene splicing, or transcript stability (21). A protective effect of the variant G allele at intron 5-5229 of the *Cox-2* gene in colorectal and prostate cancer may indicate a transcription regulatory role of intronic sequences. Alternatively, the intron 5-5229 polymorphism may be in linkage disequilibrium with a nearby functional polymorphism.

Another polymorphism with a protective effect for adenoma development was detected at position 10935 in the 3' flanking region of the *Cox-2* gene. This is located downstream of the polyadenylation signal and AU-rich elements. Previously, disease-associated variants have been described in the 3' flanking region of genes that affect transcription factor-binding sites (22). Polymorphisms in the *Cox-2* gene located upstream of the 3'FR-10935 position have been reported to have cancer-modulating effect. Especially, both positive and negative association of the 3' UTR-8494 polymorphism with various types of cancer has been widely reported (12). In particular, the heterozygous, but not the variant homozygous genotype at 3' UTR-10494 was found to be protective for prostate cancer in a Swedish population (20). Although, there was no evidence of a risk-modulating effect of the previously reported 8494 or 10494 variants of the *Cox-2* gene in our small study, the protective effect of the nearby 3' FR-10935 polymorphism underscores the significance of allelic variants in the 3' regulatory region of the *Cox-2* gene in affecting the risk of cancer development.

In summary, our study underscores the relevance of genetic variants in the regulatory regions of *Cox-2* in modulating cancer risk. In the absence of any information on the functional significance of the intron 5-5229 and 3'FR-10935 polymorphisms in development of colorectal adenoma in African Americans, future studies with larger numbers of cases and controls will be necessary to rule out the possibility that the protective effect of *Cox-2* variants in the regulatory regions on adenoma development is a chance finding.

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