**COX-2** promoter polymorphisms and the association with prostate cancer risk in South African men

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Cyclooxygenase-2 (COX-2) converts arachidonic acid to prostaglandins, which are important mediators of cell proliferation and inflammation. Evidence indicates that COX-2 plays a role in carcinogenesis and that it is over-expressed in prostate tumours. We investigated the role of COX-2 variants in prostate cancer in a case-control study of South African Coloured men, consisting of 151 cases and 134 controls. The genotype frequencies of four single-nucleotide polymorphisms (SNPs) in the COX-2 promoter were initially determined in 50 control subjects. One SNP, rs20417 (−899G>C), was monomorphic and excluded from further investigation. Three SNPs, rs3918304 (−1285A>G), rs20415 (−1265C>T) and rs5270 (−297G>C), were genotyped in all the case patients and control subjects. The −1285 G-allele and −1265 T-allele were associated with increased risk of prostate cancer [odds ratio (OR) = 3.53; confidence interval (CI) = 2.14–5.90; \( P < 0.0001 \) and OR = 3.01; CI = 1.82–5.02; \( P < 0.0001 \)] after adjusting for age. Haplotype GTC conferred increased risk of prostate cancer in South African Coloured men (OR = 3.54 versus ACC; CI = 2.12–5.92; \( P < 0.0001 \)). These findings in conjunction with findings in other populations of African descent might suggest a common causal variant for prostate cancer in COX-2, or a variant in a nearby gene.

**Introduction**

Cyclooxygenase-2 (COX-2) is an inducible enzyme that converts arachidonic acid to prostaglandins, which play a role in cell proliferation and are potent mediators of inflammation (1). Furthermore, COX-2 has been postulated to influence carcinogenesis by inhibiting apoptosis (2), inducing angiogenesis (3) and by chronic activation of COX-2 has been postulated to influence carcinogenesis by inhibiting apoptosis (2), inducing angiogenesis (3) and by chronic activation of inflammation. Evidence indicates that COX-2 plays a role in carcinogenesis and that it is over-expressed in prostate tumours. We investigated the role of COX-2 variants in prostate cancer in a case-control study of South African Coloured men, consisting of 151 cases and 134 controls. The genotype frequencies of four single-nucleotide polymorphisms (SNPs) in the COX-2 promoter were initially determined in 50 control subjects. One SNP, rs20417 (−899G>C), was monomorphic and excluded from further investigation. Three SNPs, rs3918304 (−1285A>G), rs20415 (−1265C>T) and rs5270 (−297G>C), were genotyped in all the case patients and control subjects. The −1285 G-allele and −1265 T-allele were associated with increased risk of prostate cancer [odds ratio (OR) = 3.53; confidence interval (CI) = 2.14–5.90; \( P < 0.0001 \) and OR = 3.01; CI = 1.82–5.02; \( P < 0.0001 \)] after adjusting for age. Haplotype GTC conferred increased risk of prostate cancer in South African Coloured men (OR = 3.54 versus ACC; CI = 2.12–5.92; \( P < 0.0001 \)). These findings in conjunction with findings in other populations of African descent might suggest a common causal variant for prostate cancer in COX-2, or a variant in a nearby gene.

**Materials and methods**

**Study population**

Unrelated male subjects from the South African Coloured ethnic group (18) were enrolled in the case-control study to determine genetic risk factors for prostate cancer that was conducted between the years 2004 to 2007. The study population comprising 151 cases [mean (range) age: 69 (47–88) years] with histologically confirmed prostate cancer were all from the Western Cape Province of South Africa and had undergone radical prostatectomy, transurethral resection of the prostate or prostatic biopsy at the Department of Urology, Tygerberg Hospital (Cape Town, South Africa). Controls were selected among subjects admitted to the same hospital during the same period and comprised of 134 individuals that were matched for ethnicity and age [mean (range) age: 65 (52–91) years] and were from the same geographical region. The selection criteria of age, ethnic-, geographical- and institutional-matching increase the likelihood that the controls were representative of the source population of cases.

Blood samples were collected from each subject. Clinical characteristics including prostate-specific antigen, Gleason grade, tumour node metastasis stage, age at diagnosis and family history were obtained from medical records. All controls had prostate-specific antigen levels <2.5 ng/ml and a normal digital rectal examination. All subjects were informed and gave written consent to participate in the study and allow their biological samples to be genetically analyzed, according to the Helsinki declaration. The study was approved by the Stellenbosch University, Faculty of Health Sciences, Committee for Human Research.

**SNP genotyping**

Genomic DNA was extracted from whole blood using a QIAamp DNA Kit (Qiagen, GmbH, Hilden, Germany). We used ABI 3100 Multiplex SNPshot™ Primer extension analysis (Applied Biosystems, Foster City) to screen SNPs in an 1156 bp region in the COX-2 promoter that starts 125 bp upstream of the transcription start site. The outer primer set for amplification of the 1156 bp fragment were COX-2promF5’-CTCATAGTCTCCTGCCGAG3’ and COX-2promR5’-CTCCTTCTCCCTCTCCTG3’. The respective primers for the primer extension analysis were −1285 F 5’-CCATCTAATTTACCTGCTATC3’ and −1265 R 5’-GGTATTGGGGGTATTTT3’ (reverse extension direction in this study). −899 F 5’-TTAGGACCTGTATATATAGGAGAATTTACCTT3’ and −297 F 5’-CAGCTTGTGGGTTCGATTCTT3’. We determined the allele frequency for each SNP in a panel of 50 control subjects. © The Author 2008. Published by Oxford University Press. All rights reserved. For Permissions, please email: permissions@oxfordjournals.org 2347
Among our controls, SNP rs20417 was monomorphic and it was subsequently removed from further genotype analysis.

To determine whether population stratification might be a confounding factor leading to spurious associations (19), genotype analysis was performed in a group of 65 cases and 65 controls for 24 independent polymorphic SNPs that were not in linkage disequilibrium with COX-2.

Statistical analyses
Genotype and allele frequencies were calculated for each SNP. Each polymorphism was tested in the controls to confirm that they were in Hardy–Weinberg equilibrium and linkage disequilibrium between polymorphisms was assessed. For each SNP, logistic regression was used to estimate prostate cancer–genotype odds ratios (ORs), 95% confidence intervals (CIs) and corresponding P-values adjusted for age. Logistic regression was combined with an expectation maximization algorithm to infer haplotype frequencies and assess the association between prostate cancer and specific haplotypes. Haplotype analysis inferred a probability distribution of specific haplotypes for each individual. From these, we estimated haplotype frequencies for the group (pooled), as well as separate case and control frequencies. Prostate cancer ORs (and 95% CIs) for specific haplotypes compared with the reference (highest frequency) haplotype were estimated (20). P-values for prostate cancer–haplotype association were also determined. The OR and P-values are all based on a single model, so that they are all adjusted for each other. Analyses were done in R, a language and environment for statistical computing, freely available from http://www.R-project.org. The R packages genetics, LDheatmap and haplo.stats were used.

Results
All three SNPs were in Hardy–Weinberg equilibrium among the controls (P > 0.05). A significant association with prostate cancer was observed for the −1285AG/GG (OR = 3.53; CI = 2.14–5.90; P < 0.0001) and −1265CT/TT genotype (OR = 3.01; CI = 1.82–5.02; P < 0.0001) after adjusting for age (Table I). The −297 CG genotype showed an increased risk of prostate cancer (OR = 2.56; CI = 0.98–7.58), although this result was not significant after adjustment for age (P = 0.0673) (Table I). No −297 GG genotype was observed in any of the subjects screened in the present study (Table I). As expected, there was highly significant linkage disequilibrium between the SNP pair −1285/−1265 (Figure 1).

Our study panel is considered to be a present-day homogeneous population (21), despite historically having received genetic contributions from different ancestral populations (18). We genotyped a sub-group of 65 cases and 65 controls for a panel of 24 unlinked SNPs in an attempt to address the issue of possible population stratification as a confounding factor leading to spurious associations. To test for stratification across the 24 loci, we calculated the sum of the 24 allelic frequencies across the 24 loci, we calculated the sum of the 24 allelic frequencies across the 24 loci, we calculated the sum of the 24 allelic frequencies across the 24 loci, we calculated the sum of the 24 allelic frequencies across the 24 loci, we calculated the sum of the 24 allelic frequencies across the 24 loci, we calculated the sum of the 24 allelic frequencies across the 24 loci. We then used an inflation factor leading to spurious associations (data not shown). Additionally, the P-values we obtained for our significant results were much smaller than any of those found for our unlinked independent panel of 24 SNPs, further indication that our results are probably true.

Three haplotypes of −1285A>G, 1265C>T and −297C>G had inferred frequencies of ≥5% in the study groups (Table II). Five haplotypes with individual frequencies <5% in the study groups were grouped together for the analysis (Table II). The global P-value for the fit of the logistic regression model of prostate cancer case–control status was highly significant (P < 0.0001). Haplotype GTC, compared with the haplotype ACC, was associated with prostate cancer in South African Coloured men (OR = 3.54; CI = 2.12–5.92) (Table II). Highly significant (P < 0.0001 after inflating) differences in haplotype frequencies between cases and controls were found for both haplotypes ACC (52% in cases and 73% in controls) and GTC (31% in cases and 16% in controls) (Table II).

<table>
<thead>
<tr>
<th>SNP</th>
<th>Genotype</th>
<th>Number of subjects (%)</th>
<th>OR (95% CI)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cases (%)</td>
<td>Controls (%)</td>
<td></td>
</tr>
<tr>
<td>rs3918304</td>
<td>−1285A&gt;G</td>
<td>AA</td>
<td>48 (32)</td>
<td>85 (63)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AG</td>
<td>91 (60)</td>
<td>46 (34)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GG</td>
<td>12 (8)</td>
<td>3 (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AG and GG</td>
<td>103 (68)</td>
<td>49 (36)</td>
</tr>
<tr>
<td>rs20415</td>
<td>−1265C&gt;T</td>
<td>CC</td>
<td>47 (31)</td>
<td>76 (57)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CT</td>
<td>94 (62)</td>
<td>52 (39)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TT</td>
<td>10 (7)</td>
<td>6 (4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CT and TT</td>
<td>104 (69)</td>
<td>58 (43)</td>
</tr>
<tr>
<td>rs5270</td>
<td>−297C&gt;G</td>
<td>CG</td>
<td>16 (11)</td>
<td>6 (4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GG</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

ND, not determined; OR, 95% CI and P-value could not be estimated because the observed counts in both cases and controls are zero.
*Adjusted for age. 

Fig. 1. Pairwise linkage disequilibrium analysis using D’.
A significant association was observed with the
C0
state cancer was identified with the
SNP was excluded from this investigation, an increased risk of pros-
 haplotype.
P with prostate cancer. The
promoter variants were shown to be associated
increased
in prostate tumours (8,9,25,26), although one study suggested that
South African Coloured men.
 haplotype could be protective against developing prostate cancer in
controls than in the cases. This finding could possibly suggest that this
 haplotype occurred with a significantly higher frequency in the con-
veloping prostate cancer by 3.54-fold in South African Coloured men
study demonstrated that the GTC haplotype increases the risk of de-
(14). However, our study differs marginally because the
COX-2
promoter that are associated with prostate cancer risk in South African
and prostate cancer has been shown in African American (14,16)
where the
C0
variants and prostate cancer in Caucasian men.
and concomitantly, these studies reported reduced
COX-2
dominantly of Khoi and San (African) inhabitants, with genetic con-
numerous investigations have shown that sub-Saharan, West and
investigations should be undertaken on additional
COX-2
were selected only provide a limited coverage of
African and Caucasian). A limitation of the study is that the SNPs that
it should be researched in other South African ethnic groups (Black

**Discussion**

The present study identified two SNPs and a haplotype in the
COX-2 promoter that are associated with prostate cancer risk in South African
Coloured men. A previous study reported an increased association
between prostate cancer and having the
A-allele and
C-allele and an inverse association with the
G-allele (14). In our
study, we found that the
A-allele increased the risk of prostate cancer, which confirms the association described by Panguluri
et al. (14). However, our study differs marginally because the
C-allele was excluded from this investigation, an increased risk of pro-
state cancer was identified with the
G-allele and no statistically
significant association was observed with the
G-allele. Our
study demonstrated that the GTC haplotype increases the risk of de-
veloping prostate cancer by 3.54-fold in South African Coloured men
compared with the ACC haplotype. We also noted that the ACC
haplotype occurred with a significantly higher frequency in the con-
trols than in the cases. This finding could possibly suggest that this
 haplotype could be protective against developing prostate cancer in
South African Coloured men.

The promoter region upstream of the
COX-2 transcriptional start site contains multiple putative transcription factor-binding sites
(1,24). Several studies have described an elevated
COX-2 expression in prostate tumours (8,9,25,26), although one study suggested that
increased
COX-2 levels may suppress tumour development (27). In
the present study, two promoter variants were shown to be associated with prostate cancer. The
A-allele variant does not alter a pro-
moter transcription factor site, whereas the
A-allele puta-
tively eliminates a CCAAT/enhancer-binding protein (C/EBP) γ site
and creates a Pit-1α- and Hb-binding site (14). Increased levels of
C/EBPz in mouse skin carcinoma cells (28), whereas another study demonstrated that
expression of
COX-2 is associated with decreased levels of
C/EBPz in rat hepatocytes (29). The third polymorphism analyzed in this study
(−297T>G) has been shown to create a C/EBP delta (δ)- and a ribosomal DNA-binding site (14). Interestingly, increased
COX-2 expression in mouse skin carcinogenesis has been correlated with decreased levels of
C/EBPs and concomitantly increased
COX-2 expression (28). At present, it is not yet known whether the elimina-
tion of a
C/EBPs site and creation of a
C/EBPs site may independ-
ently or synergistically alter the expression of
COX-2.

Numerous investigations have shown that sub-Saharan, West and
Central African and African American populations are related genetic-
ically (30,31), and suggestions have been made that common suscept-
ibility alleles are probably present across different African populations (32). Increased genetic risk between
COX-2 variants and prostate cancer has been shown in African American (14,16)
and Nigerian men (14); concomitantly, these studies reported reduced risk between
COX-2 variants and prostate cancer in Caucasian men. High incidence rates of prostate cancer have been detected in South African Coloured men (33), a unique ethnic group descendent predominantly of Khoi and San (African) inhabitants, with genetic con-
tributions from European settlers (predominantly Dutch, German and
French) and Asian (Indonesian and Madagascan) migrants who in-
habited the Western Cape Province of South Africa in the late 1600s
(18). Taken together, the genetic association described in this Southern African population in conjunction with findings in other populations of African descent (14,16) might suggest a common causal variant for prostate cancer in
COX-2 or a variant in a nearby gene.

Our study has a number of weaknesses and strengths. The sample
size was relatively small, although it was sufficient to detect small to
moderate associations. Our control group was closely matched to the
cases by age, ethnicity, geography and medical institution to reduce the likelihood of spurious association due to population stratification.
When we tested a panel of cases and controls for 24 independent
SNPs, we observed significant stratification between the groups. How-
ever, even after genomic control inflation of our
P-values, the results remained highly significant. The genetic association was detected in
a male population of mixed African, European and Asian descent and it should be researched in other South African ethnic groups (Black
African and Caucasian). A limitation of the study is that the SNPs that
were selected only provide a limited coverage of
COX-2. Further investigations should be undertaken on additional
COX-2 SNPs, on variants in genes flanking
COX-2 and in other genes in the inflammatory
pathway.

In conclusion, we have detected a significant genetic association
between variants in
COX-2 and prostate cancer risk in South African
men. These findings provide supporting evidence to the link between inflammation and prostate cancer. Genotype analyses of additional
gene variants are warranted and the influence of the
COX-2 promoter variants on gene expression requires investigation.

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the urologists who facilitated the collection of samples. Even though the work
is supported by the Medical Research Council, the views and opinions ex-
pressed are not those of the Medical Research Council but of the authors of the
material produced or publicized.

**Conflict of Interest Statement:** None declared.

**References**

1. Smith,W.L. et al. (2000) Cyclooxygenases: structural, cellular, and mole-
epithelial cells overexpressing prostaglandin endoperoxide synthase 2.

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**Table II.** Inferred haplotype frequencies, joint ORs and joint
P-values for tests of association with prostate cancer for each inferred haplotype in South African Coloured men

<table>
<thead>
<tr>
<th>Haplotype frequency</th>
<th>Cases</th>
<th>Controls</th>
<th>OR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>−1285A&gt;G</td>
<td>−1265C&gt;T</td>
<td>−297C&gt;G</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A C C</td>
<td>0.52</td>
<td>0.73</td>
<td>1.00 (reference)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>A T C</td>
<td>0.05</td>
<td>0.06</td>
<td>1.30 (0.64–2.64)</td>
<td>0.5714</td>
</tr>
<tr>
<td>G T C</td>
<td>0.31</td>
<td>0.16</td>
<td>3.54 (2.12–5.92)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Rare*</td>
<td>0.12</td>
<td>0.05</td>
<td>3.72 (1.82–7.61)</td>
<td></td>
</tr>
</tbody>
</table>

Global

*Haplotypes with joint/pooled frequencies <5% were grouped together. No P-value is given for prostate cancer-rare haplotype association, because it is not a single haplotype.


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