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 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 (−1265G>T) and rs5270 (−297G>C), were genotyped in all the case and control subjects. The −1285G allele and −1265T 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 inflammation. Evidence indicates that COX-2 plays a role in carcinogenesis and 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 (−1265G>T) and rs5270 (−297G>C), were genotyped in all the case and control subjects. The −1285G allele and −1265T 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.

Abbreviations: C/EBP, CCAAT/enhancer-binding protein; CI, confidence interval; COX-2, cyclooxygenase-2; OR, odds ratio; SNP, single-nucleotide polymorphism.

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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, genotyping 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 possible 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 genoStats, 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 −1285 AG/GG (OR = 3.53; CI = 2.14–5.90; P < 0.0001) and −1265 CT/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 subgroup 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 association χ2 statistics (22), which gave a total of 54.0 at 24 degrees of freedom (P = 0.0004), indicating that significant stratification exists between the cases and controls included in our study. The median χ2 value for comparison of allele frequencies between cases and controls, among the 24 SNPs, was 1.39 (P = 0.3557), resulting in an inflation factor of λ = 1.39/0.4549 = 3.06 (23). All our results remained significant when we inflated our P-values by multiplying them with the inflation factor (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 to 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).

![Fig. 1. Pairwise linkage disequilibrium analysis using D’.

### Table I. Genotype frequencies and ORs for prostate cancer among COX-2 promoter SNPs in South African Coloured men

<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>rs3918304</td>
<td>−1285A&gt;G</td>
<td>AA 48 (32) GG 85 (63)</td>
<td>1.00 (reference)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AG 91 (60) GG 46 (34)</td>
<td>3.39 (2.04–5.73)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GG 12 (8) GG 3 (2)</td>
<td>5.52 (1.59–25.72)</td>
<td>0.0130</td>
</tr>
<tr>
<td>rs20415</td>
<td>−1265C&gt;T</td>
<td>CC 47 (31) TT 76 (57)</td>
<td>1.00 (reference)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CT 94 (62) TT 52 (39)</td>
<td>3.15 (1.89–5.34)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TT 10 (7) TT 6 (4)</td>
<td>1.88 (0.59–6.36)</td>
<td>0.2920</td>
</tr>
<tr>
<td>rs5270</td>
<td>−297C&gt;G</td>
<td>CC 135 (89) TT 128 (96)</td>
<td>1.00 (reference)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GG 16 (11) GG 6 (4)</td>
<td>2.56 (0.98–7.58)</td>
<td>0.0673</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.
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 −1265 A-allele and −899 C-allele and an inverse association with the −297 G-allele (14). In our study, we found that the −1265 T-allele increased the risk of prostate cancer, which confirms the association described by Panguluri et al. et al. (14). However, our study differs marginally because the −899G>C SNP was excluded from this investigation, an increased risk of prostate cancer was identified with the −1285 G-allele and no statistically significant association was observed with the −297 G-allele. Our study demonstrated that the GTC haplotype increases the risk of developing 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 controls 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 −1285A>G variant does not alter a promoter transcriptional factor site, whereas the −1265 A-allele putatively eliminates a CCAAT/enhancer-binding protein (C/EBP) α site and creates a Pit-1α- and Hb-binding site (14). Increased levels of C/EBPα have been shown to down-regulate COX-2 expression in mouse skin carcinoma cells (28), whereas another study demonstrated that up-regulation of COX-2 is associated with decreased levels of C/EBPα in rat hepatocytes (29). The third polymorphism analyzed in this study (−297C>G) has been shown to create a C/EBP 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/EBPα and concomitantly increased C/EBF6 expression (28). At present, it is not yet known whether the elimination of a C/EBPα site and creation of a C/EBF6 site may independently 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 genetically (30,31), and suggestions have been made that common susceptibility alleles are probably to be 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 descendant predominantly of Khoi and San (African) inhabitants, with genetic contributions from European settlers (predominantly Dutch, German and French) and Asian (Indonesian and Madagascan) migrants who inhabited 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. However, 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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Conflict of Interest Statement: None declared.

References


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>−1285A&gt;G</th>
<th>−1265C&gt;T</th>
<th>−297C&gt;G</th>
<th>Haplotype frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>C</td>
<td>C</td>
<td>0.52</td>
</tr>
<tr>
<td>A</td>
<td>T</td>
<td>C</td>
<td>0.05</td>
</tr>
<tr>
<td>G</td>
<td>T</td>
<td>C</td>
<td>0.31</td>
</tr>
<tr>
<td>Rare*</td>
<td></td>
<td></td>
<td>0.12</td>
</tr>
</tbody>
</table>

G Halldorsson frequencies, joint ORs and joint P-values are given for prostate cancer-rare haplotype association, because it is not a single haplotype.

Global P-value for model <0.0001.

*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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