Null Results in Brief

No Association between Common Chemokine and Chemokine Receptor Gene Variants and Prostate Cancer Risk

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Abstract

There is growing evidence that inflammation and infection play important roles in the etiology of prostate cancer. As the chemokine network is directly involved in inflammation and infectious diseases, we tested for an association between six common putative functional variants and prostate cancer risk using an Australian case-control study. We measured *CCL5* −403G>A, *CXCL12* +801G>A, *CCR2*V64I (G>A), *CCR5*Δ32, *CX3CR1*V249I (G>A), and *CX3CR1*T280M (C>T) for 815 cases and 738 controls. Of these, only *CXCL12* +801G>A has previously been tested and found to be associated with prostate cancer risk. We found no significant associations with prostate cancer risk (all $P > 0.4$). All per allele odds ratios ranged from 0.96 (95% confidence intervals, 0.80-1.16) to 1.06 (95% confidence intervals, 0.90-1.23). This suggests that these common chemokine and chemokine receptor variants do not play a major, if any, role in susceptibility to prostate cancer. (Cancer Epidemiol Biomarkers Prev 2008;17(12):3615–17)

Introduction

Inflammation and infection are thought to play a major role in the pathogenesis of prostate cancer (1), fuelling studies into associations between genetic variation in inflammatory genes and prostate cancer risk (2, 3). A subset of cytokines involved in the regulation of inflammation and shown to modulate tumor behavior are those involved in the chemokine network (4). We hypothesize that functional variants within genes of this network could result in a balance shift of the chemokine and chemokine receptor system towards a favorable angiogenic, antiapoptotic, or metastatic response, promoting the initiation and development of prostate cancer. To test our hypothesis, we screened six commonly studied genetic variants previously shown to have a measurable functional effect on the corresponding protein (5–12). These include CC chemokine ligand 5 (*CCL5*, MIM#187011) −403 promoter variant (rs2107538G>A), CXC chemokine ligand 12 (*CXCL12*, MIM#600835) +180 3′ untranslated regulatory variant (rs1801157G>A), CC chemokine receptor 2 (*CCR2*, MIM#601267) nonsynonymous variant *CCR2*V64I (rs1799864G>A), chemokine receptor 5 (*CCR5*, MIM#601373) 32 bp deletion variant *CCR5*Δ32, and CX3C chemokine receptor (*CX3CR1*, MIM#601470) nonsynonymous variants *CX3CR1*V249I (rs3732379G>A) and *CX3CR1*T280M (rs3732378C>T). The *CXCL12* +801G>A polymorphism was recently shown to be a risk factor for prostate cancer in a Japanese study (13). The remaining variants, although associated with many infectious diseases and cancers, have not yet been investigated in prostate cancer.

Materials and Methods

Study Population. We tested our hypotheses in the Australian Risk Factors for Prostate Cancer Study, a population-based case-control study including 815 cases and 738 controls predominantly of European descent (14). Eligible cases were newly diagnosed adenocarcinomas of the prostate notified to the State Cancer Registry during 1994 to 1997 and histopathologically confirmed. Cases were excluded if age at diagnosis was ≥70 years or if tumor was well-differentiated (Gleason score < 5). Cases were classified into tumor stage groupings according to the American Joint Committee on Cancer

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Table 1. Allele and genotype distribution of *CCL5, CXCL12, CCR2, CCR5* and *CX3CR1* polymorphisms in 829 prostate cancer cases and 739 controls from the Australian Risk Factors for Prostate Cancer Study

Gene polymorphism*	NCBI ID	Minor allele frequencies (n) †		Per allele OR (95% CI) ‡	P§	Heterozygous OR (95% CI)‖	Homozygous OR (95% CI)‖	P (recessive)¶	P (dominant)**
		Cases	Control						
CCL5 −403 (G/A)	rs2107538	0.17 (801)	0.18 (727)	0.96 (0.80-1.16)	0.70	0.89 (0.71-1.12)	1.18 (0.68-2.03)	0.47	0.46
CXCL12 +801 (G/A)	rs1801157	0.20 (815)	0.19 (727)	1.05 (0.88-1.24)	0.60	1.06 (0.85-1.33)	1.06 (0.67-1.69)	0.86	0.57
*CCR2*V64I (G/A)	rs1799864	0.08 (813)	0.08 (738)	1.02 (0.79-1.32)	0.86	0.98 (0.74-1.30)	1.59 (0.46-5.46)	0.45	0.98
*CCR5*Δ32	—	0.11 (815)	0.11 (727)	1.00 (0.80-1.25)	0.98	1.00 (0.78-1.29)	0.97 (0.43-2.22)	0.95	0.99
*CX3CR1*V249I (G/A)	rs3732379	0.29 (808)	0.27 (730)	1.06 (0.90-1.23)	0.49	1.05 (0.85-1.30)	1.12 (0.77-1.62)	0.62	0.54
*CX3CR1*T280M (C/T)	rs3732378	0.17 (805)	0.18 (728)	0.99 (0.82-1.19)	0.89	0.98 (0.79-1.23)	0.98 (0.54-1.77)	0.96	0.88

*The second base pair indicates the minor allele.
† Minor allele frequency and actual number for cases and controls.
‡ ORs and 95% CIs from unconditional logistic regression analysis for the additive model.
§Test for association between genotype and prostate cancer risk for the additive model (likelihood ratio test).
‖ORs and 95% CIs from unconditional logistic regression analysis for the codominant model. ORs are relative to homozygotes for the common allele.
¶Test for association between genotype and prostate cancer risk for the recessive model (likelihood ratio test).
**Test for association between genotype and prostate cancer risk for the dominant model (likelihood ratio test).

definition. Controls were randomly selected from the State Electoral Rolls and frequency matched to cases by 5-year age group and city of residence.

Genotyping. TaqMan allelic discrimination assays (Applied Biosystems) were used to genotype single nucleotide polymorphisms, *CCL5* −403G>A, *CXCL12* +801G>A, *CCR2*V64I (G>A), *CX3CR1*V249I (G>A), and *CX3CR1*T280M (C>T), whereas gel-based amplicon size differentiation was used for screening the deletion *CCR5*Δ32 (protocols available on request). Randomly, 20% of the study was replicated with 100% concordance.

Statistical Analyses. Allele frequency estimates and tests of deviation from Hardy-Weinberg equilibrium were carried out using standard procedures based on asymptotic likelihood theory (15). Pairwise linkage disequilibrium between variants was assessed using Lewontin's D′ and r-squared (r^2), estimated using R (16).[7] Fisher's exact test was used to test for independence between the single nucleotide polymorphisms and age (<55, 55-64, 65-69) and country of birth (Australia, others). Polytomous logistic regression models were used to estimate odds ratios (OR) by tumor stage (stage I-II and stage III-IV tumors), grade (moderate grade and high grade tumors), and family history of prostate cancer (affected first-degree relative and two or more first-degree relatives affected). Tests for association between genotypes and case-control status were done under dominant, recessive, codominant, and additive models. Case-control analyses were conducted using unconditional logistic regression (17) and OR estimates and their 95% confidence intervals (CI) were derived under likelihood theory using Stata/SE 8.2 (Stata Corporation). All tests were two-sided and 5% level was used as a threshold for statistical significance.

Results

Allele frequencies for controls ranged from 8% (*CCR2*V64I) to 27% (*CX3CR1*V249I) and were similar or identical to allele frequencies for cases (Table 1). Genotype frequencies were consistent with Hardy-Weinberg equilibrium for both cases and controls (all $P > 0.05$), whereas no association with age (all $P > 0.05$) was observed. Carriers of the rare allele for *CCR5*Δ32 were more prevalent in men born in Australia (23% versus 15%, $P = 0.006$) whereas carriers of the rare allele for *CCR2*V64I were less prevalent in men born in Australia (14% versus 20%, $P = 0.03$).

*CX3CR1*V249I and *CX3CR1*T280 were in almost perfect linkage disequilibrium (D′ = 1.00, $P < 0.001$, $r^2 = 0.54$ for cases and controls combined). The remaining variants, including those in the *CX3CR1, CCR5*, and *CCR2* genes that are in close proximity (18), were not linked (all D′ < 0.15).

We found no evidence of association between any of the selected genetic variants and prostate cancer risk (all $P > 0.4$). The estimated per allele ORs ranged from 0.96 (95% CI, 0.80-1.16) for *CCL5* −403 to 1.06 (95% CI, 0.90-1.23) for *CX3CR1*V249I. No OR was significantly different in any of the recessive, dominant, codominant, or additive models. Adjustment for known measured risk factors (age of diagnosis, family history of prostate cancer, and country of birth) did not significantly change the OR estimates. ORs did not differ by tumor stage or grade (all $P > 0.05$). For *CCR5*Δ32, ORs increased with the number of first degree-relatives affected ($P = 0.04$) with ORs per allele being 0.92 (95% CI, 0.73-1.17), 1.22 (95% CI, 0.82-1.82), and 2.30 (95% CI, 1.13-4.68) for none, one, and two or more first degree-relatives affected, respectively. The ORs did not differ by family history of prostate cancer for the remaining variants (all $P > 0.05$).

Discussion

This is the largest case-control study to explore associations between common functional chemokine network variants and risk for prostate cancer. The relatively large sample size provides adequate statistical power (80% or higher at 0.05 significance level) to detect moderate or strong associations (ORs ≥ 1.60). We cannot, however, rule out modest associations. We excluded late-onset cases (diagnosis > 69 years) and cases with well-differentiated tumors (Gleason scores of ≥5) to identify genetic variants associated with clinically significant disease. Another strength of our study is the

[7] http://www.r-project.org/

population-based design with age-matched controls being recruited from the general population using the Electoral Rolls.

CXCL12 +801G>A is the only variant previously investigated for association with prostate cancer risk. A small Japanese case-control study of 167 cases and 167 age-matched controls reported that ORs heterozygous and homozygous for the rare allele relative to homozygous for the common allele were 1.34 (95% CI, 0.63-2.86) and 1.58 (95% CI, 1.03-2.43), respectively (5). We did not find evidence of association with both ORs being equal to 1.06. A likely explanation of the difference between the Japanese study and our own is chance. Other possible explanations are genotyping method used (indirect gel-based screening versus automated), characteristics of cases (earlier onset versus more aggressive), and selection of controls (volunteers screened for prostate cancer at a single hospital versus random selection of population and age-matched controls). Although the allele frequency of *CXCL12* +801G>A in the Japanese controls (27%) is higher than in our controls (19%), there is no obvious reason to assume that the association with prostate cancer risk is population-specific (19).

This study is the first comprehensive analysis of common *CCL5, CXCL12, CCR2, CCR5,* and *CX3CR1* biologically functional variants and prostate cancer risk. Overall, our results suggest that these genetic variants do not play a major role in susceptibility to prostate cancer. However, the observed association between *CCR5*Δ32 and familial prostate cancer risk (cases with two or more first-degree relatives affected) may warrant further independent investigations.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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