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Short Communication

Interleukin-10 [ATA] promoter haplotype and prostate cancer risk: A population-based study

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ABSTRACT

Interleukin-10 (IL-10) is a multifunctional cytokine acting as inhibitor of inflammatory and immune responses as well as tumour induced angiogenesis. A common [ATA] haplotype formed by polymorphisms at positions −1082, −819 and −592 in the promoter of the IL-10 gene is a strong determinant for IL-10 expression. The presence of this haplotype can be determined by analysis of the −592C > A polymorphism. To analyse the role of the IL-10 [ATA] haplotype in prostate cancer we performed a case-control study including 561 prostate cancer patients and 561 male, age-matched, control subjects without malignant disease. The IL-10 −592C > A polymorphism was determined by a 5′-nuclease assay (TaqMan). IL-10 −592 CC, CA and AA genotype frequencies were not significantly different between patients (53.6%, 40.0%, 6.4%) and controls (54.3%, 39.6%, 6.1%; $p = 0.96$). IL-10 genotypes were furthermore not associated with tumour characteristics such as histological grade, T stage, PSA levels at diagnosis, or age at diagnosis. Therefore we conclude that the IL-10 −592C > A promoter polymorphism, tagging the IL-10 low-producer [ATA] haplotype, is not associated with risk for prostate cancer.

1. Introduction

Prostate cancer is the most commonly diagnosed cancer in western men and its incidence is rising rapidly in most countries.[1] Currently, age, ethnicity and a family history of prostate cancer are the only established risk factors for this disease.[2] Evidence on diet, especially animal fat intake, is mounting but inconclusive.[3] Furthermore, chronic inflammation may be associated with carcinogenesis due to DNA damage through radical oxygen and nitrogen species.[4] Data on other risk factors, such as circulating levels of hormones, physical activity, body size, smoking, drinking, sexual behaviour and occupational exposures, are conflicting.[2]

Environmental factors alone, however, cannot fully explain the individual differences in prostate cancer risk. The evidence that prostate cancer has a genetic component is compelling from epidemiological and genetic studies. Some risk gene variants have been identified, which may predispose carriers to development of disease.[5] Yet more common, lower penetrance susceptibility polymorphisms in genes may

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be implicated in a higher portion of the sporadic prostate cancer disease burden.

Interleukin-10 (IL-10) is a multifunctional TH-2 cytokine participating in the development and progression of various tumours.[6] It was originally discovered as a powerful inhibitor of inflammatory and immune responses, partly via its inhibition of some macrophage responses. IL-10 can also inhibit tumour-induced angiogenesis, and a wealth of evidence is accumulating that IL-10 carries some immuno-stimulating properties.[7]

In prostate cancer, overexpression of IL-10 has been reported.[8] The dual biological function of IL-10 may reflect the conflicting evidence on the role of IL-10 in cancer development and progression. As an immunosuppressive molecule allowing malignant cells to escape from immune surveillance, it may act as a potential tumour promoter resulting in a more aggressive behaviour of tumour cells.[9,10] On the other hand, the immune-stimulating and antiangiogenic effects of IL-10, partly via down-regulation of MMP-2, are supposed to prevent or reduce tumour growth and distant tumour spread.[11]

Three common polymorphisms in the promoter of the IL-10 gene (−1082G > A, −819C > T, −592C > A] show strong linkage disequilibrium and form two common haplotypes, designated as [ATA] and [GCC] haplotype.[12,13] The [ATA] haplotype has been associated with lower IL-10 expression in several studies.[14,15] Due to a strong linkage disequilibrium, the presence of this haplotype can be fully determined by analysis of the −592C > A polymorphism (rs1800872). The −592A allele indicates the presence of the [ATA] haplotype, whereas the −592C allele indicates its absence.[14]

In the present study we evaluated the role of the IL-10 [ATA] promoter haplotype for prostate cancer in a large case control study.

2. Patients and methods

Between January 2004 and November 2005, 561 male patients with sporadic, histologically confirmed prostate cancers were recruited at the Department of Therapeutic Radiology and Oncology, Medical University of Graz. Clinical characteristics including prostate specific antigen (PSA) at the time of diagnosis, tumour node metastasis (TNM) stage, tumour grade, furthermore age at diagnosis, family history and means of diagnosis were obtained from medical records. The median time between diagnosis of prostate cancer and study entry was 14 months.

For each patient, one healthy male age-matched control subject was included. Controls were selected from a local population-based screening study ($n = 184$). Additional controls ($n = 377$) were recruited from patients admitted to the Department of Internal Medicine for other reasons than malignant disease. Subjects with any current or previous malignant disease were not eligible as control subjects.

The study was performed according to the Austrian Gene Technology Act and has been approved by the Ethical Committee of the Medical University of Graz. Written informed consent was obtained from all participating subjects. All subjects were Caucasian.

IL-10 genotypes were determined by a 5′-exonuclease assay (TaqMan).[16] Primer and probe sets were designed and manufactured using Applied Biosystems 'Assay-by-Design' custom service (Applera, Austria). The PCR was performed in a Primus 96 plus thermal cycler (MWG Biotech AG, Germany) using a total volume of 5 μl containing 2.5 μl Super-Hot- Master-Mix (Bioron GmbH, Germany), 0.125 μl Assay- by-design Mix (Applera), 0.375 μl H_2O and 2 μl DNA. Reactions were overlaid with 15 μl mineral oil. Cycling parameters were: 1 min 94° for primary denaturation, followed by 40 cycles of 15 s 92° and 1 min 60°. Fluorescence was measured in a Lambda Fluoro 320 Plus plate reader (MWG Biotech AG, Germany) using excitation/emission filters of 485/ 530 nm for FAM-labeled probes (IL-10 −592A allele) and 530/572 nm for VIC-labeled probes (IL-10 −592C allele). The data were exported into Excel format and analysed as scatter plot. As a quality control, 95 samples were reanalysed, results were identical for all samples.

Statistic analysis was done using SPSS 11.0 for Windows. Numeric values were analysed by Student's t-test, proportions of groups were compared by χ^2-test. Odds ratio (OR) was calculated to estimate the risk for prostate cancer. Threshold for significance was $p < 0.05$.

3. Results

IL-10 genotypes were successfully determined in 545 (97.1%) control subjects and 547 (97.5%) prostate cancer patients. In the remaining subjects, no DNA was available or genotypes were considered non-interpretable after three repeats.

IL-10 −592C > A genotypes did not deviate from the Hardy–Weinberg equilibrium among patients or controls. Demographic and genetic data are summarised in Table 1. The IL-10 −592C > A polymorphism was not associated with prostate cancer risk and the odds ratio of the −592A allele for prostate cancer was 1.03 (0.85–1.25). The IL-10 polymorphism was furthermore not associated with tumour characteristics (Table 2).

The present study had a statistical power of 0.80 to detect an odds ratio of 1.4 or higher for carriers of the IL-10 −592A variant.

4. Discussion

The IL-10 promoter contains several polymorphisms showing strong linkage disequilibrium. A common [ATA] haplotype is formed by polymorphisms at positions −1082, −819 and −592.[12,13] Lower IL-10 expression has been reported for the −592-A variant,[14] the −1082-A variant[15] as well as for the [ATA] haplotype.[16] Thus, the −592-A variant can be regarded as a low-producer allele of the IL-10 gene.

In the present study, allele and genotype frequencies of the IL-10 −592C > A polymorphism, tagging a common functional haplotype, were not different between prostate cancer patients and healthy controls, suggesting that this genetic marker is not a risk factor for prostate cancer. The IL-10 −592C > A polymorphism was not associated with tumour size, histological grade, PSA levels at time of diagnosis, or age at diagnosis.

The strengths of our study include the relatively large number of cases and controls, resulting in a high statistical

Table 1 – Demographic and genetic data of control subjects and prostate cancer patients

	Controls subjects	Prostate cancer patients	P-value
Patient number	545	547	–
Age, years	68.0 ± 7.1	68.0 ± 7.1	(matching criteria)
IL-10 −592			
CC	296 (54.3)	293 (53.6)	0.96
CA	216 (39.6)	219 (40.0)	
AA	33 (6.1)	35 (6.4)	
IL-10 −592-A frequency	0.259	0.264	0.77

Data are presented as number (percentage), or mean ± standard deviation. P-values were determined by the Chi-square-test.

Table 2 – IL-10 −592C > A genotypes and tumour characteristics

		CC	CA	AA	P-value
Histological grade, n (%)	1	27 (9.2)	20 (9.2)	5 (14.3)	0.40
	2	171 (58.6)	117 (53.7)	15 (42.9)	
	3	94 (32.2)	81 (37.2)	15 (42.9)	
T (stage), n (%)	1–2	147 (54.9)	109 (52.7)	19 (59.4)	0.75
	3–4	121 (45.1)	98 (47.3)	13 (40.6)	
PSA levels, median (range)	ng/ml	0.7 (0–154.8)	0.5 (0–204.9)	0.5 (0–13.1)	0.37
Perineural invasion, n (%)	No	210 (77.8)	156 (74.3)	26 (78.8)	0.64
	Yes	60 (22.2)	54 (25.7)	7 (21.2)	
Lymphatic vessel invasion, n (%)	No	254 (93.7)	196 (93.3)	29 (87.9)	0.45
	Yes	17 (6.3)	14 (6.7)	4 (12.1)	
Age at diagnosis, mean ± sd	Years	68.2 ± 6.4	67.7 ± 8.0	68.0 ± 7.4	0.80

power. Furthermore, prostate cancer patients and controls were sex- and age-matched and from an ethnic homogenous population.

Nevertheless, it should be taken into account that IL-10 levels were not determined, thus the findings of the present study are valid primarily for IL-10 genotypes and do not disprove a role for the cytokine itself.

Our finding is in line with a previous large Swedish case-control study by Xu et al. who also did not observe an association between the −592C > A polymorphism (denoted alternatively as −627C > A) and prostate cancer.[17] In another population-based study dealing with the same topic, Michaud and co-workers investigated the influence of three IL-10 polymorphisms (rs1800896, rs1800871, rs3024496) on the risk for prostate cancer development.[18] Although the −592C > A polymorphism was not analysed in that study, the work of Michaud and co-workers suggests that the IL-10 promoter polymorphisms do not contribute to prostate cancer susceptibility.

McCarron and colleagues reported an increased risk for prostate cancer in carriers of the homozygous IL-10 1082 AA genotype.[19] As had been stated by the authors, their result was presented as preliminary and was not confirmed in a subsequent much larger investigation.[21]

A number of methodical papers on genetic association studies have stressed the importance of studies confirming (or confuting) results of primary reports.[20,21] Replication of association studies is imperative to draw firm conclusions about the role of genetic risk factors, but association studies with negative results are sometimes misjudged as 'failure' and held back from publication. We are convinced that the communication of negative results, if they result from well powered and methodically sound studies, is important for a clear and unbiased view of the relevance of genetic markers.

Summarising the findings from the present study and previous reports, it can be concluded that carriage of IL-10 low-producer or high-producer haplotypes does not contribute to prostate cancer susceptibility.

Conflict of interest statement

None declared.

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