Prostate Cancer and Prostatic Diseases (2013) 16, 56 – 60

www.nature.com/pcan

ORIGINAL ARTICLE

Genetic factors regulating inflammation and DNA methylation associated with prostate cancer

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BACKGROUND: Prostate cancer (PCa) displays a strong familiarity component and genetic factors; genes regulating inflammation may have a pivotal role in the disease. Epigenetic changes control chromosomal integrity, gene functions and ultimately carcinogenesis. The enzyme glycine-*N*-methyltransferase (*GNMT*) contributes to S-adenosylmethionine level regulation and, by affecting DNA methylation, influences gene expression. The genotype and allele distribution of single-nucleotide polymorphisms (SNPs) in the promoter regions of vascular endothelial growth factor (*VEGF*), interleukin (*IL*)-*10*, *IL-1*β, alpha-1-antichymotrypsin (*ACT*) and *GNMT* genes, the level of global DNA methylation and the influence of *GNMT* SNP upon DNA methylation in a PCa case–control study have been investigated.
METHODS: SNPs of *VEGF* (rs699947), *ACT* (rs1884082), *IL-1*β (rs16944), *IL-10* (rs1800896) and *GNMT* (rs9462856) genes were assessed by PCR or by real-time PCR methods. DNA methylation was assessed by an ELISA assay.
RESULTS: Frequencies of the *VEGF* AA genotype, the *IL-10* A allele and *GNMT* T allele were higher in PCa. The concomitant presence of the AA genotype of *VEGF*, the A allele of *IL-10* and T allele of *GNMT* increased the risk of PCa. Total DNA methylation was decreased in PCa; control *GNMT* T carriers (T+) showed the highest level of DNA methylation.
CONCLUSIONS: SNPs in *VEGF*, *IL-10* and *GNMT* genes might have a synergistic role in the development of PCa. The *GNMT* T allele may influence PCa risk by affecting DNA methylation and prostate gene expression. Our observations might help implement the screening of unaffected subjects with an increased susceptibility to develop PCa.

Prostate Cancer and Prostatic Diseases (2013) **16,** 56–60; doi:10.1038/pcan.2012.30; published online 31 July 2012

Keywords: cytokine gene polymorphism; DNA methylation; prostate cancer risk

INTRODUCTION

Prostate cancer (PCa) is one of the most relevant cancers in the elderly and represents 4% of male cancer in developing countries and 15% in industrialized societies.[1] PCa shows a clinical progression and contributes to morbidity and mortality in men.[2] PSA assays resulted in an increased detection and clinical incidence of PCa. The Randomized European Study for PCa[3] revealed that PSA was associated with 20% relative reduction in the death rate at a median follow-up of 9 years. However, the clinical efficacy of PSA screening is still conflicting. Therefore, the search for other genetic and phenotypic markers able to further define the risk of PCa in non-symptomatic subjects is of great interest.

Infections and inflammatory responses are linked to 15–20% of all deaths from cancers worldwide; chronic inflammation has been suggested to have a pivotal role in the pathogenesis of PCa,[4] and is now linked to early steps of carcinogenesis and cancer progression.[5] Chronic prostatic inflammation is involved in the development and progression of PCa.[6,7] Cancer-related inflammation is characterized by the presence of leukocytes and inflammatory mediators paralleling the chronic inflammation usually observed in tissue remodeling and repair.[5] Cytokines are major regulators of inflammation and genetic cytokine markers have been investigated in PCa. Single-nucleotide polymorphisms (SNPs) of interleukin *(IL)-1*β, *IL-8* and *IL-10* genes have been shown to affect the risk of PCa.[8] However, a subsequent investigation did not confirmed the above data.[9] A recent meta-analysis of *IL-10* SNPs in PCa showed that some *IL-10* SNPs affected cancer progression whereas other SNPs were modestly associated with advanced stages of PCa.[10] Factors affecting angiogenesis might have a relevant role in tumor growth and progression[5] and conflicting results regarding the association of SNP in the vascular endothelial growth factor (*VEGF*) gene with PCa have been reported.[11–15] Therefore, the association between SNPs in candidate genes with modulatory activities on inflammation and PCa remains an open question.

Tumor-associated macrophages by producing reactive oxygen derivatives may induce DNA damage and mutations.[16] Inflammation induces aberrant tissue epigenetic alterations involved in carcinogenesis, and the imbalance of cytokine and chemokine release influences tissue abnormal DNA methylation.[17]

Altered methylation in selected genes has been recently reported in PCa.[18,19] A metabolomic profile investigation in patients with PCa has shown an increment of urinary sarcosine in PCa clinical progression.[20] Sarcosine is an *N*-methyl derivative of glycine and glycine-*N*-methyltransferase (*GNMT*) generates sarcosine from glycine and regulates *S*-adenosylmethionine levels.[21] *GNMT*, therefore, is a metabolic link between the *de novo* synthesis of methyl groups and the availability of dietary methionine. *GNMT*, by affecting DNA methylation, influences genetic stability. *GNMT* expression is decreased in PCa tissue and the loss of heterozygosity in the *GNMT* promoter region is associated with PCa.[22] Pathological epigenetic changes have gained relevance as mechanisms controlling chromosomal integrity, gene functions and carcinogenesis.[23] To the best of our knowledge, formal investigations of germ line genetic variation in genes controlling

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Received 24 February 2012; revised 5 June 2012; accepted 24 June 2012; published online 31 July 2012

methylation and its relationship with inflammatory markers associated to PCa risk have not been investigated.

We studied the SNP genotype and allele distribution of several genes with inflammatory modulatory activity such as *VEGF*, *IL-10*, *IL-1*β, alpha-1-antichymotrypsin (*ACT*). *IL-10* and *IL-1*β gene SNPs were selected for their role in suppressing or promoting inflammation.[5] *ACT* is an acute phase protein and a protease inhibitor that binds PSA.[24] The *VEGF* gene is a relevant factor controlling angiogenesis.[5] The *GNMT* enzyme influences DNA methylation by affecting *de novo* synthesis of methyl groups.[21] All investigated SNPs are located in the promoter region and affect the expression of the cognate gene.[25] The level of global DNA methylation in PCa and control leukocytes was also investigated.

MATERIALS AND METHODS

Patients and controls

During 2008–2009 screening, at the Urology Department of Fidenza Hospital (Italy), patients were scheduled for prostate biopsy because of abnormal rectal digital examination findings, a PSA level $>3\,ng\,ml^{-1}$ and % free PSA $<22\%$. Those patients ($n=244$ mean age 69 ± 6 years) with a positive prostate biopsy underwent radical prostectomy for PCa and were included in this study.

The Gleason grade score was assigned according to the World Health Organization/International Society of Urological Pathologist Consensus Conference Criteria.[26]

Lymph node surgery was performed on the basis of preoperative prognostic factors and all the surgical specimens were histopathologically evaluated.

The control population belonged to the Conselice Study of Brain Aging from Northern Italy,[27] and included 259 healthy men subjects (mean age 77 ± 6 years) with no inflammatory and cardiovascular diseases or history of prostate alterations. Controls consisted of elderly males belonging to a longitudinal population study followed up for 5 years. This selection was intended to minimize the presence among controls of carriers with early onset subclinical PCa.

DNA extraction

To reduce cost and facilitate patient enrollment in the genetic/epigenetic study, DNA samples from PCa patients were obtained using buccal swabs (Epicentre Biotechnologies, San Diego, CA, USA). DNA extraction from swab was performed by adding 600 μl of 50 mM NaOH to brush and heating at 95 °C for 5 min. After brush removal, 60 μl of 1 M Tris, pH 8, was added. Genomic DNA was obtained by spinning the sample at 13 000 rpm for 1 min.

DNA control samples were obtained from peripheral blood leukocytes, as previously described.[28]

SNP detection

SNPs in the promoter regions of *VEGF* (−2578 C/A; rs699947) and *ACT* (−51 G/T; rs1884082) were assessed by a PCR-based method (MJ TERMAL CYCLER) described elsewhere.[28,29]

SNPs in the *IL-1*β (−511 C/T; rs16944) and *IL-10* (−1082 G/A; rs1800896) genes were detected by real-time PCR method. SNP-specific primers and probes were designed according to the TaqMan genotyping assay by ABI (Foster City, CA, USA) and assays were performed in 25 μl total volume on Bio-Rad CFX 96 (Hercules, CA, USA), following manufacturer's instructions, as previously described.[25]

The SNP in the promoter at position −2476 (rs9462856) of *GNMT* gene consisted of a C→T transversion. Patients and controls were genotyped by PCR DNA amplification using primer pairs: 5′-AGCTCATG ATCCACCTCAGC-3′/ 5′-CACAGCCTTCCCTGCATTAT-3′ with 5 min at 95 °C for the initial denaturation and 29 cycles of 30 s at 94 °C, 30 s at 52 °C and 30 s at 72 °C. Then, 4-min incubation at 72 °C for final extension was performed. The restriction enzyme Bstz 171 (MBI Fermentas, Italy; 10 U/sample) resolved the DNA amplification product in three different band patterns, according to the three different genotypes; for example, at 97 and 147 bp (CC genotype), 97, 147 and 250 bp (CT genotype) or 250 bp (TT genotype).

Quality controls of genotyping were assessed by sequencing randomly selected DNA samples using Beckman CEQ2000 (Fullerton, CA, USA). Genotype and allele determination was obtained in each individual.

DNA methylation detection

Global DNA methylation detection was performed using the Methylamp Global DNA Methylation Quantification Ultra Kit (Epigentek, Farmingdale, NY, USA). Following the manufacturer's prescriptions, sensitivity was as low as 0.2 ng of methylated DNA and the universal positive control was suitable for quantifying methylated DNA from any species. Briefly, DNA samples were immobilized to strip wells specifically coated with DNA affinity substance. The methylated fraction of DNA was recognized by 5-methylcytosine antibody and quantified through an ELISA-like reaction; the amount of methylated DNA resulted proportional to the optical density (OD) intensity.

For an accurate calculation of DNA methylation, OD experimental sample values vs amount of a positive control were plotted, and the slope as OD per ng was calculated according to the following formula:

$$\text{Methylated DNA (ng)} = \text{Sample OD} - \text{negative Control}/\text{Slope}.$$

Absorbance was recorded by an automatic ELISA reader at 450 nm (Bio-Rad, Milan, Italy).

PSA detection

Serum samples were obtained before any diagnostic procedure. Total immune reactive and free PSA were assessed by the chemiluminescence immune assay (Immulite: Diagnostic Products, Los Angeles, CA, USA) using an automated analyzer (detection limits: 0.02 and $0.03\,mg\,l^{-1}$ for free PSA and total PSA). Percentage of free PSA was calculated as the ratio of free PSA to total PSA in %.[30]

Statistical analysis

Statistical analysis of continuous variables from the controls and PCa patients was performed by one-way analysis of variance with appropriate *post hoc* comparisons using Fisher's test and Bonferroni (two-tailed analysis) correction, and statistical significance was set at 0.05 level. The presence of the Hardy–Weinberg equilibrium for allele frequency of each SNP in control population was verified.

The genotype distribution and allele frequency were compared with contingency tables and chi square (χ^2) analysis. Odds ratio (OR) and confidence intervals (CI 95%) for PCa risk were also calculated.

To evaluate SNP interaction with epidemiological variables associated to the PCa risk, a logistic regression model was used. Statistical analysis was assessed by using the SPSS 11.01 software package (SPSS, Chicago, IL, USA).

RESULTS

PCa TNM evaluation showed a *t* score $=2$ in 82 and *t* score $=3$ in 83; average Gleason's score was 7 ± 1 s.d. and average blood PSA was 8 ± 5 s.d. ($\mu g\,ml^{-1}$).

Genotype distributions and allele frequencies in *VEGF*, *ACT*, *IL-1*β, *IL-10* and *GNMT* SNPs were studied. The departure from Hardy–Weinberg equilibrium in controls was tested. *ACT*, *IL-1*β, *IL-10* and *GNMT* SNPs were in equilibrium, and their allele frequencies were comparable to those found in HapMap, as shown in NCBI database. Only the *VEGF* SNP slightly departed from the Hardy–Weinberg equilibrium ($P=0.02$), and the selection criteria applied to the control population (for example, gender, age, no inflammatory and cardiovascular diseases) may explain this result.

The *VEGF*, *ACT*, *IL-1*β, *IL-10* and *GNMT* genotype distribution and allele frequency between PCa and controls have been reported in Table 1. The *VEGF* AA genotype frequency was higher in PCa than in controls (PCa = 24.6%, controls = 15.4%) and the difference

Table 1. Genotype and allele distribution of SNP in the promoter region of the *VEGF*, *ACT*, *IL-1*, *IL-10* and *GNMT* genes from PCa patients and controls

VEGF	*CC* (n) %[a]	*CA* (n) %	*AA* (n) %	*C carrier* (n) %	*A carrier* (n) %
PCa ($n=224$) %	(115) 51.3	(54) 24.1	(55) 24.6	(168) 75.0	(109) 48.7
Controls ($n=156$) %	(75) 48.1	(57) 36.5	(24) 15.4	(132) 84.6	(81) 51.9
$\chi^2=8.779$, $P=0.012$					
$\chi^2=4.695$, $P=0.03$, OR = 1.790, Genotype AA (CI = 1.053–3.043)					
$\chi^2=5.115$, $P=0.024$, Allele C					
$\chi^2=0.391$, $P=0.532$, Allele A					
ACT	GG[a]	GT	TT	G carrier	T carrier
PCa ($n=184$)	(49) 26.6	(90) 48.9	(45) 24.5	(139) 75.5	(135) 73.4
Controls ($n=256$)	(59) 23	(128) 50	(69) 27	(186) 72.7	(197) 77
$\chi^2=0.843$, $P=0.656$					
$\chi^2=0.462$, $P=0.497$, Allele G					
$\chi^2=0.742$, $P=0.389$, Allele T					
IL-1β	CC[a]	CT	TT	C carrier	T carrier
PCa ($n=78$)	(43) 55.1	(30) 38.5	(5) 6.4	(72) 93.5	(37) 48.1
Controls ($n=259$)	(123) 47.5	(106) 40.9	(30) 11.6	(229) 88.4	(136) 52.5
$\chi^2=2.345$, $P=0.310$					
$\chi^2=1.648$, $P=0.199$ Allele C					
$\chi^2=0.472$, $P=0.492$, Allele T					
IL-10	GG[a] (n) %	GA (n) %	AA (n) %	G carrier (n) %	A carrier (n) %
PCa ($n=171$) %	(18) 10.5	(74) 43.3	(79) 46.2	(91) 53.5	(153) 89.5
Controls ($n=96$) %	(28) 29.2	(43) 44.8	(25) 26	(74) 77.1	(65) 67.7
$\chi^2=18.846$, $P=0.0001$					
$\chi^2=14.452$, $P=0.0001$, Allele G					
$\chi^2=19.438$, $P=0.000$, OR = 4.054, Allele A (CI = 2.118–7.760)					
$\chi^2=10.505$, $P=0.001$, OR = 2.439 Genotipo AA (CI = 1.413–4.210)					
GNMT	CC[a] (n) %	CT (n) %	TT (n) %	C carrier (n) %	T carrier (n) %
PCa ($n=244$) %	(55) 22.5	(142) 58.2	(47) 19.3	(197) 80.7	(189) 77.5
Controls ($n=217$) %	(69) 31.8	(101) 46.5	(47) 21.7	(170) 78.3	(148) 68.2
$\chi^2=6.941$, $P=0.031$					
$\chi^2=0.406$, $P=0.524$, Allele C					
$\chi^2=5.005$, $P=0.025$, OR = 1.602, Allele T (CI = 1.054–2.425)					

[a]Wild-type genotype.
Abbreviations: ACT, alpha-1-antichymotrypsin; CI, confidence interval; GNMT, glycine-*N*-methyltransferase; IL, interleukin; OR, odds ratio; PCa, prostate cancer; SNP, single-nucleotide polymorphism; VEGF, vascular endothelial growth factor.

was statistically significant ($P=0.03$; OR = 1.790, CI = 1.053–3.043). On the contrary, the percentage of the C allele was significantly increased in controls (controls = 84.6% vs PCa = 75%, $P=0.024$; OR = 0.545, CI = 0.321–0.926).

ACT and *IL-1*β genotype distribution and allele frequency were comparable in the two populations.

The *IL-10* AA genotype was more frequent in PCa than in controls (PCa = 46.2% vs controls = 26%, $P=0.001$; OR = 2.439 CI = 1.413–4.210). The percentage of A carriers was overrepresented in PCa than in controls (PCa = 89.5% vs controls = 67.7%, $P=0.0001$; OR = 4.054, CI = 2.118–7.760).

The *GNMT* T allele frequency was higher in PCa than in controls (PCa = 77.5% vs controls = 68.2%; $P=0.025$; OR = 1.602, CI = 1.058–2.425).

The concomitant presence of the AA genotype of *VEGF*, the A allele of *IL-10* and the T allele of *GNMT* in patients and controls was also determined (Table 2). This genetic signature, here called 'triple genotype', was more represented in PCa than in controls (PCa = 18.2%, controls = 7.2%, $P=0.003$; OR = 2.878, CI = 1.409–5.880). Therefore, 'triple genotype' carriers showed an increased risk of PCa.

Statistical analysis adjusted for a potential confounding variable such as cigarette smoking was performed by logistic regression analysis, and the AA *VEGF* ($P=0.001$, OR = 2.993) and *IL-10* ($P=0.001$, OR = 11.454) genotypes, along with the *GNMT* T allele ($P=0.004$, OR = 1.765), were found to be independently associated with the risk of PCa.

Table 2. Concomitant presence of the AA genotype of *VEGF*, the A allele of *IL-10* and the T allele of *GNMT* genes (triple genotype) in patients with PCa and controls

Triple genotype	*Carrier*	*Not carrier*
PCa ($n=192$) %	(35) 18.2	(157) 81.8
Controls ($n=153$) %	(11) 7.2	(142) 92.8
$\chi^2=8.980$, $P=0.003$, OR = 2.878, CI = 1.409-5.880		

Abbreviations: CI, confidence interval; GNMT, glycine-*N*-methyltransferase; IL, interleukin; PCa, prostate cancer, OR, odds ratio; VEGF, vascular endothelial growth factor.

Quantification of global genomic DNA methylation from peripheral blood leukocytes was performed (Table 3). Differences in nanograms of total methylated DNA between PCa (mean = 6.66 ± 2.38) and controls (mean = 8.47 ± 2.83) were detected

Table 3. Global DNA methylation in patients with PCa and controls and the influence of the *GNMT* T allele on DNA methylation in patients and controls

Clinical and GNMT carrier status	n	*Mean of ng methylated DNA*	*s.d.*
PCa	38	6.66	2.38
Controls	25	8.47	2.83
PCa T+	18	7.20	2.16
PCa T−	20	6.17	2.52
Controls T+	11	9.52	3.02
Controls T−	14	7.64	2.48
Statistical analysis of methylated DNA:			
PCa vs controls, $F = 7.44$ $P = 0.008$			
PCa T+ vs T− vs controls, T+ vs T−, $F = 4.29$ $P = 0.008$			
PCa T+ vs controls T+ $F = 5.806$ $P = 0.023$			

Abbreviations: GNMT, glycine-*N*-methyltransferase; PCa, prostate cancer.

($P = 0.008$). After stratifying subjects in *GNMT* T carriers (T+) and non-carriers (T−), differences in methylated DNA between the four groups of PCa, T+ (mean = 7.20 ± 2.16) and T− (mean = 6.17 ± 2.52), controls T+ (mean = 9.52 ± 3.02) and T− (mean = 7.64 ± 2.48) were found ($F = 4.29$, $P = 0.008$). In particular, a lower methylated DNA in PCa *GNMT* T+ than in controls *GNMT* T+ was present ($F = 5.806$, $P = 0.023$). On the other hand, no statistically difference of methylated DNA between PCa *GNMT* T− and controls *GNMT* T– was observed ($F = 2.817$, $P = 0.103$).

DISCUSSION

Chronic inflammation is a risk factor for initiation and progression of PCa.[31,32] Therefore, SNPs in the promoter region of several genes with inflammatory regulatory role in this study were investigated and the AA genotype of *VEGF* and *IL-10* genes resulted as genetic risk factors for the disease. Our data were in accordance with other investigations, showing that SNPs in the *VEGF* gene were associated with an increased risk of PCa.[11,13] However, other studies did not support a role of *VEGF* SNP in PCa.[12,14] Our data regarding *IL-10* SNP association in PCa are in accordance with other case–control investigations.[33,34] Other studies, however, were not able to confirm any association of *IL-10* SNPs with the disease.[35] Discrepancies among different case–control studies might be related to patient/control selection, ethnic heterogeneity or differential PCa stages. Our patients showed a restricted range of the T and Gleason Scores. PCa and controls were ethnically homogenous, as they were Caucasian and from a restricted region of northern Italy. Controls were selected to minimize the presence of subclinical and early-onset prostate diseases (even if they were not formally screened for PCa), and consisted of elderly individuals with no clinical history of prostate pathology or cardiovascular and inflammatory diseases before and during a follow-up period of 5 years. Our previous study[25] showed that (rs699947) VEGF SNP was associated with an increased risk of acute myocardial infarction. Therefore, the selection of male controls according to advanced age, no prostate and cardiovascular diseases might explain the slight departure of Hardy–Weinberg equilibrium only for this SNP.

It is known that the A allele of both *VEGF* and *IL-10* SNPs were associated with an impaired production of the cognate protein.[36,37] Our results support the notion that subjects with a genetic make up favoring an impaired control of inflammatory responses may be at risk for PCa.

The T allele of the rs9462856 SNP in the promoter region of the *GNMT* gene was overrepresented in patients with PCa and increased the risk of the disease. Our data parallel those from another study showing that (1) haplotypes of *GNMT* polymorphism, defined as GA repeats, were associated with a differential risk of PCa, that (2) cellular transfection with the haplotype C (10GAs/Ins/T) reporter gene exhibited the highest promoter activity and that (3) *GNMT* expression was downregulated in cancer tissue from PCa patients.[22]

The concomitant presence of AA genotype of the *VEGF* gene, the A allele of the *IL-10* gene and the T allele of the *GNMT* gene ('triple genotype') was increased in patients with PCa, and subjects positive for the triple genotype were at high risk for the disease. The 'triple genotype' might have a synergistic role in the development of PCa and used to identify unaffected subjects with elevated risk of developing PCa.

Pathological epigenetic changes are gaining increasing relevance as novel mechanisms controlling chromosomal integrity and gene functions.[23] DNA hypomethylation by inducing generation of chromosomal instability contributes to cancer development.[38] On the other hand, promoter hypermethylation, by inhibiting tumor suppressor genes, may promote carcinogenesis.[23]

It is interesting to note that PCa patients showed a decreased global DNA methylation and patients positive for the *GNMT* T allele had a lower methylated DNA level than controls with the same allele. DNA hypomethylation in PCa was found by genome-wide methylation analysis[39] and by immunohistochemistry method using a monoclonal antibody specific for 5-methylcytosine.[40] Therefore, the T allele of *GNMT* SNP might influence PCa risk by affecting DNA methylation of pro-inflammatory genes, which in turn may control cancer progression.

DNA samples from PCa patients were obtained from buccal swabs, whereas those from controls were obtained from blood leukocytes. The DNA source difference does not affect SNP genotyping, as gene variations are equally distributed in all nucleated cells, but might affect epigenetic changes. However, leukocytes are the prevalent cellular components of saliva samples.[41] DNA profiling from buccal swabs of patients receiving allogeneic peripheral blood stem cells showed that the DNA pattern of graft recipients partially overlapped that from donors of allogeneic stem cells.[42] Therefore, blood leukocytes appear to be the prevalent component of buccal swab, and our epigenetic data may be mainly referred to this cells.

CONCLUSIONS

Aging is a risk factor for PCa,[2] epigenetic DNA modifications are observed during cell senescence[43] and total genomic methylation correlates with the maximum life span.[44] The age-associated epigenetic drift may be related to differences in environmental exposure,[45] as supported by the observation that twin pairs who have spent less of their lifetime together showed increased epigenetic differences.[43] We speculate that the aberrant inflammatory mechanisms by affecting DNA methylation, particularly in carriers of the triple genotype, might result in further impaired inflammatory responses. Decreased DNA methylation from PCa patients may be secondary to an increased activation of these cells by inflammatory cytokines. Inflammation has been shown to affect survival of patients with PCa,[46] and the *IL-10* AA genotype influenced PSA recurrence in patients after radical prostatectomy.[47]

In conclusion, genes with a regulatory function on inflammation and affecting the one-carbon metabolism may have a synergistic role in the susceptibility of PCa. Genomic and epigenetic data obtained in this preliminary investigation need to be replicated in a larger case–control study to fully substantiate the validity of our present findings.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank Dr Stefania Bitonte for technical assistance. We thank Dr Christine M Betts for kindly grammatical editing the manuscript.

Funding: Research partially supported by Italian Pallotti Grants and Cassa di Risparmio Foundation in Bologna.

REFERENCES

1 Quinn M, Babb P. Patterns and trends in prostate cancer incidence, survival, prevalence and mortality. Part I: international comparisons. *BJU Int* 2002; **90**: 162–173.
2 Holman CD, Wisniewski ZS, Semmens JB, Rouse IL, Bass AJ. Mortality and prostate cancer risk in 19 598 men after surgery for benign prostatic hyperplasia. *BJU Int* 1999; **84**: 37–42.
3 Schröder FH, Hugosson J, Roobol MJ, Tammela TL, Ciatto S, Nelen V *et al.* Screening and prostate-cancer mortality in a randomized European study. *N Engl J Med* 2009; **360**: 1320–1328.
4 Koul HK, Kumar B, Koul S, Deb AA, Hwa JS, Maroni P *et al.* The role of inflammation and infection in prostate cancer: importance in prevention, diagnosis and treatment. *Drugs Today (Barc)* 2010; **46**: 929–943.
5 Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. *Nature* 2008; **454**: 436–444.
6 De Nunzio C, Kramer G, Marberger M, Montironi R, Nelson W, Schröder F *et al.* The controversial relationship between benign prostatic hyperplasia and prostate cancer: the role of inflammation. *Eur Urol* 2011; **60**: 106–117.
7 Omabe M, Ezeani M. Infection, inflammation and prostate carcinogenesis. *Infect Genet Evol* 2011; **11**: 1195–1198.
8 McCarron SL, Edwards S, Evans PR, Gibbs R, Dearnaley DP, Dowe A *et al.* Influence of cytokine gene polymorphisms on the development of prostate cancer. *Cancer Res* 2002; **62**: 3369–3372.
9 Michaud DS, Daugherty SE, Berndt SI, Platz EA, Yeager M, Crawford ED *et al.* Genetic polymorphisms of interleukin-1B (IL-1B), IL-6, IL-8, and IL-10 and risk of prostate cancer. *Cancer Res* 2006; **66**: 4525–4530.
10 Shao N, Xu B, Mi YY, Hua LX. IL-10 polymorphisms and prostate cancer risk: a meta-analysis. *Prostate Cancer Prostatic Dis* 2011; **14**: 129–135.
11 Sfar S, Saad H, Mosbah F, Chouchane L. Combined effects of the angiogenic genes polymorphisms on prostate cancer susceptibility and aggressiveness. *Mol Biol Rep* 2009; **36**: 37–45.
12 Jain L, Vargo CA, Danesi R, Sissung TM, Price DK, Venzon D *et al.* The role of vascular endothelial growth factor SNPs as predictive and prognostic markers for major solid tumors. *Mol Cancer Ther* 2009; **8**: 2496–2508.
13 VanCleave TT, Moore JH, Benford ML, Brock GN, Kalbfleisch T, Baumgartner RN *et al.* Interaction among variant vascular endothelial growth factor (VEGF) and its receptor in relation to prostate cancer risk. *Prostate* 2010; **70**: 341–352.
14 Langsenlehner T, Langsenlehner U, Renner W, Krippl P, Mayer R, Wascher TC *et al.* Single nucleotide polymorphisms and haplotypes in the gene for vascular endothelial growth factor and risk of prostate cancer. *Eur J Cancer* 2008; **44**: 1572–1576.
15 Jacobs EJ, Hsing AW, Bain EB, Stevens VL, Wang Y, Chen J *et al.* Polymorphisms in angiogenesis-related genes and prostate cancer. *Cancer Epidemiol Biomarkers Prev* 2008; **17**: 972–977.
16 Solinas G, Germano G, Mantovani A, Allavena P. Tumor-associated macrophages (TAM) as major players of the cancer-related inflammation. *J Leukoc Biol* 2009; **86**: 1065–1073.
17 Niwa T, Ushijima T. Induction of epigenetic alterations by chronic inflammation and its significance on carcinogenesis. *Adv Genet* 2010; **71**: 41–56.
18 Kelavkar UP, Harya NS, Hutzley J, Bacich DJ, Monzon FA, Chandran U *et al.* DNA methylation paradigm shift: 15-lipoxygenase-1 upregulation in prostatic intra-epithelial neoplasia and prostate cancer by atypical promoter hypermethylation. *Prostaglandins Other Lipid Mediat* 2007; **82**: 185–197.
19 Pierconti F, Martini M, Pinto F, Cenci T, Capodimonti S, Calarco A *et al.* Epigenetic silencing of SOCS3 identifies a subset of prostate cancer with an aggressive behavior. *Prostate* 2011; **71**: 318–325.
20 Sreekumar A, Poisson LM, Rajendiran TM, Khan AP, Cao Q, Yu J *et al.* Metabolomic profiles delineate potential role for sarcosine in prostate cancer progression. *Nature* 2009; **457**: 910–914.
21 Luka Z, Mudd SH, Wagner C. Glycine N-methyltransferase and regulation of S-adenosylmethionine levels. *J Biol Chem* 2009; **284**: 22507–22511.
22 Huang YC, Lee CM, Chen M, Chung MY, Chang YH, Huang WJ *et al.* Haplotypes, loss of heterozygosity, and expression levels of glycine N-methyltransferase in prostate cancer. *Clin Cancer Res* 2007; **13**: 1412–1420.
23 Hatziapostolou M, Iliopoulos D. Epigenetic aberrations during oncogenesis. *Cell Mol Life Sci* 2011; **68**: 1681–1702.
24 Partin AW, Brawer MK, Bartsch G, Horninger W, Taneja SS, Lepor H *et al.* Complexed prostate specific antigen improves specificity for prostate cancer detection: results of a prospective multicenter clinical trial. *J Urol* 2003; **170**: 1787–1791.
25 Licastro F, Chiappelli M, Porcellini E, Campo G, Buscema M, Grossi E *et al.* Gene-gene and gene - clinical factors interaction in acute myocardial infarction: a new detailed risk chart. *Curr Pharm Des* 2010; **16**: 783–788.
26 Epstein JI, Allsbrook Jr WC, Amin MB, Egevad LL. The 2005 International Society of Urological Pathology (ISUP) consensus conference on gleason grading of prostatic carcinoma. *Am J Surg Pathol* 2005; **29**: 228–1242.
27 Ravaglia G, Forti P, Maioli F, Chiappelli M, Montesi F, Tumini E *et al.* Blood inflammatory markers and risk of dementia: The Conselice Study of brain aging. *Neurobiol Aging* 2007; **28**: 1810–1820.
28 Licastro F, Chiappelli M, Grimaldi LM, Morgan K, Kalsheker N, Calabrese E *et al.* A new promoter polymorphism in the alpha-1-antichymotrypsin gene is a disease modifier of Alzheimer's disease. *Neurobiol Aging* 2005; **26**: 449–453.
29 Chiappelli M, Borroni B, Archetti S, Calabrese E, Corsi MM, Franceschi M *et al.* VEGF gene and phenotype relation with Alzheimer's disease and mild cognitive impairment. *Rejuvenation Res* 2006; **9**: 485–493.
30 Benecchi L, Pieri AM, Pastizzaro CD, Potenzoni M. Evaluation of prostate specific antigen acceleration for prostate cancer diagnosis. *J Urol* 2011; **185**: 821–826.
31 Rajarubendra N, Lawrentschuk N, Bolton DM, Klotz L, Davis ID. Prostate cancer immunology - an update for urologists. *BJU Int* 2011; **107**: 1046–1051.
32 Vasto S, Carruba G, Candore G, Italiano E, Di Bona D, Caruso C. Inflammation and prostate cancer. *Future Oncol* 2008; **4**: 637–645.
33 McCarron SL, Edwards S, Evans PR, Gibbs R, Dearnaley DP, Dowe A *et al.* Influence of cytokine gene polymorphisms on the development of prostate cancer. *Cancer Res* 2002; **62**: 3369–3372.
34 Wang MH, Helzlsouer KJ, Smith MW, Hoffman-Bolton JA, Clipp SL, Grinberg V *et al.* Association of IL10 and other immune response- and obesity-related genes with prostate cancer in CLUE II. *Prostate* 2009; **69**: 874–885.
35 Michaud DS, Daugherty SE, Berndt SI, Platz EA, Yeager M, Crawford ED *et al.* Genetic polymorphisms of interleukin-1B (IL-1B), IL-6, IL-8, and IL-10 and risk of prostate cancer. *Cancer Res* 2006; **66**: 4525–4530.
36 Mateo I, Llorca J, Infante J, Rodríguez-Rodríguez E, Sánchez-Quintana C, Sánchez-Juan P *et al.* Case-control study of vascular endothelial growth factor (VEGF) genetic variability in Alzheimer's disease. *Neurosci Lett* 2006; **401**: 171–173.
37 Crawley E, Kay R, Sillibourne J, Patel P, Hutchinson I, Woo P. Polymorphic haplotypes of the interleukin-10 5′ flanking region determine variable interleukin-10 transcription and are associated with particular phenotypes of juvenile rheumatoid arthritis. *Arthritis Rheum* 1999; **42**: 1101–1108.
38 Esteller M. Epigenetics in cancer. *N Engl J Med* 2008; **358**: 1148–1159.
39 Kim SJ, Kelly WK, Fu A, Haines K, Hoffman A, Zheng T *et al.* Genome-wide methylation analysis identifies involvement of TNF-α mediated cancer pathways in prostate cancer. *Cancer Lett* 2011; **302**: 47–53.
40 Yang B, Sun H, Lin W, Hou W, Li H, Zhang L *et al.* Evaluation of global DNA hypomethylation in human prostate cancer and prostatic intraepithelial neoplasm tissues by immunohistochemistry. *Urol Oncol* 2011 (e-pub ahead of print 23 June 2011).
41 Aps JK, Van den MK, Delanghe JR, Martens LC. Flow cytometry as a new method to quantify the cellular content of human saliva and its relation to gingivitis. *Clin Chim Acta* 2002; **321**: 35–41.
42 Zhou Y, Li S, Zhou J, Wang L, Song X, Lu X *et al.* DNA profiling in blood, buccal swabs and hair follicles of patients after allogeneic peripheral blood stem cells transplantation. *Leg Med* 2011; **13**: 47–51.
43 Grolleau-Julius A, Ray D, Yung RL. The role of epigenetics in aging and autoimmunity. *Clin Rev Allergy Immunol* 2010; **39**: 42–50.
44 Wilson VL, Smith RA, Ma S, Cutler RG. Genomic 5-methyldeoxycytidine decreases with age. *J Biol Chem* 1987; **262**: 9948–9951.
45 Jaenisch R, Bird A. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nat Genet* 2003; **33** (Suppl): 245–254.
46 McArdle PA, Qayyum T, McMillan DC. Systemic inflammatory response and survival in patients with localised prostate cancer: 10-year follow-up. *Urol Int* 2010; **85**: 482.
47 Lin HC, Liu CC, Kang WY, Yu CC, Wu TT, Wang JS *et al.* Influence of cytokine gene polymorphisms on prostate-specific antigen recurrence in prostate cancer after radical prostatectomy. *Urol Int* 2009; **83**: 463–470.