Alpha 1 Antichymotrypsin Genotype is Associated with Increased Risk of Prostate Carcinoma and PSA Levels

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Abstract. *Background: Prostate cancer (PCa) is the most common non-skin cancer among men in Western countries. Inflammation appears to be involved in the pathogenesis of PCa. Recent studies have shown that many inflammatory genes are associated with the risk of PCa. Alpha 1 antichymotrypsin (ACT) is an acute phase protein and it is part of the circulating prostate specific antigen (PSA). Patients and Methods: Allele and genotype frequencies of a promoter single nucleotide polymorphism (SNP) in ACT gene were investigated in patients with benign prostate hypertrophy (BHP) or PCa and controls. Results: The G allele was more represented in PCa patients (odds ratio=2.349). The PSA levels and prostatic volume did not correlate with the ACT genotype. However, stratifying subjects by age, a correlation of PSA levels and the GG genotype in young PCa patients was found. Conclusion: Carriers of the ACT G allele are at risk of developing PCa and genotyping healthy subjects could be a new approach for early prevention.*

Prostate carcinoma (PCa), the most common non-cutaneous malignant neoplasm among men in Western countries, is responsible for the deaths of approximately 30,000 men per year in the United States (1).

A substantial number of familial PCa is caused by genetic factors, as suggested by studies from twins showing higher concordance rates for PCa in monozygotic compared to dizygotic twins (2).

It has been suggested that approximately 20% of human cancer in adults results from acute or chronic inflammatory states (3). The molecular mechanisms underlying the regulation of inflammation associated with cancer are complex, and involve both the innate and adaptive immune systems. For instance chronic inflammation may lead to tumorigenesis by damaging DNA through radical oxygen and nitrogen species or by enhancing cell proliferation and stimulating angiogenesis (3).

Emerging evidence also suggests that inflammation is a relevant pathogenetic factor for PCa. The involvement of inflammatory processes in PCa is suggested by epidemiological investigations showing a negative correlation between the use of non-steroidal anti-inflammatory drugs (NSAID) and the risk of PCa. In fact, a meta-analysis of five studies showed that the use of aspirin was associated with a 15% reduction of prostate cancer (4).

Recently, multiple genes with regulatory roles on inflammatory pathways have been associated with prostate cancer risk. These genes include ribonuclease L (*RNASEL*), macrophage scavenger receptor 1 (*MSR1*), macrophage inhibitory cytokine-1 (*MIC-1*), interleukins (*IL-8, IL-10*), vascular endothelial growth factor (*VEGF*) and intercellular adhesion molecule (ICAM) (5). Polymorphisms in innate and adaptive immune genes may affect the nature and extent of the immune response within the prostate, including the likelihood of persistent prostatic infection and chronic inflammation (6).

Many researchers have investigated the association of single nucleotide polymorphisms (SNPs) in candidate genes and have suggested that the genetic background was important. In fact many prostate cancer modifier genes could differentially affect levels of the risk for PCa. For instance, polymorphisms in inflammatory genes, such as IL-10 (–1082), IL-8 (–251) and VEGF (–1154), were found to be associated with the risk of PCa, while a polymorphism in the IL-1 gene (–511) was not (7).

Increased plasma levels of some inflammatory molecules could also influence the risk of PCa, since levels of C-reactive protein (CRP), an acute phase protein of inflammation, were increased in PCa patients (8).

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Prostate specific antigen (PSA) is commonly used for prostate cancer screening. However, increased PSA serum levels are also associated with prostate inflammatory disease. In fact, high PSA levels were found in patients with benign prostate hypertrophy (BHP) or prostatitis (9). Furthermore, it is difficult to discriminate prostate cancer from benign prostate diseases in patients with serum PSA levels of 4-10 ng/mL (grey zone). At present, the diagnosis of PCa is possible only by a biopsy.

Most circulating PSA is complexed with the protease inhibitor alpha-1-antichymotrypsin (ACT) also called SERPINA 3. ACT is a serine protease inhibitor, and an acute phase protein primarily secreted by the liver after IL-1 or IL-6 stimulation.

It has been suggested that a high local expression of ACT in PCa tissue could facilitate the formation of PSA-ACT complexes (10). The proportion of PSA-ACT is larger and the free fraction is smaller in PCa than in BHP. However, the use of the PSA-ACT complex as a marker of PCa is not universally accepted (11). The ratio of prostate specific antigen minor molecular forms to total prostate specific antigen is constant regardless of the pathological condition of the prostate (12). A previous report has demonstrated that the specificity of the PSA-ACT complex for PCa detection was greater than that of total PSA in a large multi-institutional prospective study (13). Although PSA-ACT was apparently correlated with PCa, another study did not confirm the clinical utility of detecting PSA-ACT complex values (14).

Many patients with PCa belong to the diagnostic grey zone (*i.e.* PSA=4-10 ng/ml) and the use of the PSA value or the PSA-ACT complex as diagnostic tools is under constant revision. Some patients with PCa may show very low levels of serum PSA. Therefore it is important to find new tools associated with the disease that can help identifying subjects with elevated risk of the disease. Finally, it is important to keep in mind that protein marker levels may fluctuate in patients as a function of age, diet, concomitant pathology and individual genetic background.

ACT is a unique molecule, due to its multiple functions as an anti-inflammatory factor, a protease inhibitor and a component of the PSA complex.

Here a case/control study of a SNP in the promoter region of the ACT and the association of this SNP with the risk of developing PCa is presented.

Materials and Methods

Patients and controls. Three different groups of subjects were investigated. The control population belonged to the Conselice study of brain aging (15) from Northern Italy and included about 294 healthy men subjects (mean age 77±5 years). Another independent control population of 153 men (mean age 60±7 years) from the Urology Department of S. Orsola-Malpighi Hospital,

Table I. *Clinical features of controls, benign prostate hypertrophy (BHP) subjects and prostate carcinoma patients (PCa). Values are shown as mean±standard deviation.*

	Controls	BHP	PCa
Age	60±7	63±6	67±7
PSA (ng/ml)	0.97±0.7	2.32±2.9	9.93±8.28
Volume (dl)	24.5±5.3	47.6±20.6	44.44±20.5
pT			
pT2			47.3%
pT3			52.7%
pN (57/112)			
pN+			10.5%
pN0			89.5%
Family history			
Yes	2.6%	3.0%	0.8%
No	97.4%	97.0%	99.2%
Gonorrhea			
No	100%	100%	100%
Syphilis			
Yes			3.1%
No	100%	100%	96.9%
smoking			
Yes	0.7%	1.5%	6.2%
No	99.3%	98.5%	93.8%
Vasectomy			
No	100%	100%	100%

Bologna, Italy, was also studied. Samples from 135 patients with BHP (mean age 63±6) and 137 PCa patients (mean age 67±7) were also collected from the Urology Department of S. Orsola-Malpighi Hospital.

The diagnosis of PCa followed standard criteria which included a positive prostate biopsy in patients with a clinical suspicious of PCa (total PSA>2.5 ng/ml and/or positive rectal examination and/or presence of a hypo-echo area at transrectal ultrasound of prostate). All the patients were submitted to radical prostatectomy, lymph-node surgery was performed on the basis of pre-operative prognostic factors and all the surgical specimens were histopathologically evaluated.

The diagnosis of BHP was made on the presence of lower urinary tract symptoms, a transrectal ultrasound showing a prostate volume greater than 30cc, prostate enlargement assessed by digital rectal examination and a total PSA value lower than 2.5 or between 2.5 and 10 ng/ml in patients with two prior negative biopsies with at least 8 cores taken.

DNA extraction and gene polymorphism detection. DNA extraction from peripheral blood leukocytes was assessed, as previously described (16). ACT promoter SNP at the position –51 was also assessed as previously described (16).

PSA detection. The PSA level was measured with a chemo-luminescence assay method (Roche Modular analytics E 170).

Statistical analysis. Statistical analysis of the variables from the controls, BHP subjects and patients with CPa were performed by

Table II. *Genotype distribution and allele frequency of a SNP at –51 position in the ACT gene promoter region of controls (Ctr), benign prostate hypertrophy (BHP) and prostate carcinoma (PCa) patients.*

ACT	GG		GT		TT		G allele carrier		T allele carrier	
	n	%	n	%	n	%	n	%	n	%
Ctr	102	22.8	225	50.3	120	26.8	327	73.2	345	77.2
BHP	21	15.8	80	60.2	32	24.1	101	75.9	112	84.2
PCa	38	34.2	58	52.3	15	13.5	98	86.5	73	65.8

Chi-square: 17.382, $p=0.002$; PCa *vs.* controls G allele Chi-square: 8.618, $p=0.003$, OR 2.349 (1.311-4.207); PCa *vs.* controls T allele Chi-square: 6.165, $p=0.013$, OR 0.586 (0.362-0.891).

one way ANOVA, followed by appropriate *post hoc* comparisons using the Fisher test and Bonferroni's correction.

The genotype distribution and allele frequency were compared by contingency tables and Chi-square analysis. Odds ratio (OR) and confidence intervals (ci) were also calculated and statistical significance was assessed by using the SPSS 11.01 software package.

Results

The clinical features of the controls, BHP subjects and patients with PCa are shown in Table I.

The two populations of controls were used to increase the statistical power. They did not show any difference in ACT genotype distribution or allele frequencies ($p=0.27$), data were then pooled and results are reported in Table II. The GG genotype and G allele frequencies were less represented in the controls than in the patients with PCa (PCa with G allele: $p=0.003$; OR=2.349; ci 1.311-4.207). A logistic regression analysis adjusted for age was also applied and the adjusted OR for PCa in the subjects with the GG genotype was 2.676 (ci 1.375-5.205 $p=0.004$). Conversely, the frequency of the T allele was higher in the controls than in the PCa patients (Chi-square=20.593; $p=0.0001$; OR=0.568; ci 0.362-0.891).

The PSA plasma level was higher ($p=0.0001$) in the PCa patients (9.93 ng/ml) than in the patients with BHP (2.32 ng/ml) or the controls (0.97 ng/ml).

The total PSA and prostate volume did not correlate with the ACT genotype in the controls, BHP or the patients with PCa (data not show). Patients with PCa or BHP and the controls were stratified into two age intervals. The ACT genotype affected the PSA plasma levels only in the young PCa patients (age interval: 56-65 years) (Figure 1, panel A) with the GG genotype showing higher PSA levels (23.48±18.63) than the GT or the TT genotypes (9.78±5.98 and 9.89±6.95 ng/ml, respectively; F=7.021, $p=0.014$).

The ACT genotype did not influence the PSA plasma levels in the older subjects with PCa (Figure 1, panel B; $p=0.15$).

Discussion

The role and the levels of PSA-ACT complex are not clear (14), but ACT, by acting as a protease inhibitor may bind, inactivate and favour the clearance of protease produced by the prostate.

Our data showed that a SNP in the promoter region of ACT was more represented in the patients with PCa, the G allele or GG genotype showing 2.3 or 2.7 higher fold risk of the disease. A correlation between PSA plasma levels and the ACT GG genotypes was also found in the younger patients with PCa.

Previous studies have shown that transfection of the ACT G allele, into hepatocyte cell lines, was associated with decreased gene report transcription (17). Therefore, subjects with the GG genotype may produce and/or release decreased levels of ACT and inactivate less prostate protease than individuals with the T allele or TT genotype. The decrement in ACT turnover may significantly increase (over 25%) the risk of prostate cancer.
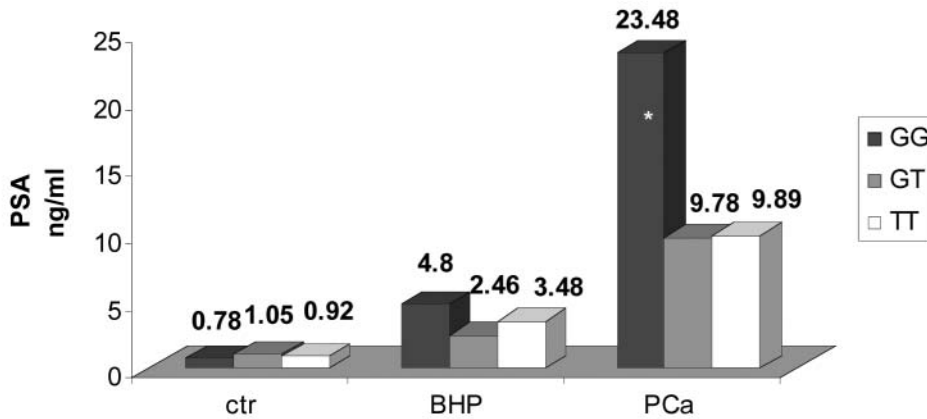
The data showing partially different age-related pathogenic pathways of the disease, with the ACT genetic components being more relevant in younger patients, reinforce the notion that ACT genotyping may be useful to identify subjects at high risk before 65 years of age.

An inflammatory condition of the prostate, such as BHP showed no differences in genotype and allele distributions in comparison with the controls, however, a weak association of the ACT GG genotype with increased PSA plasma levels was once again observed only in the young patients with BHP. These data are also in line with a decreased protective power of ACT in individuals with the ACT GG genotype.

This new genetic marker in the promoter region of the ACT gene appears to be informative for identifying subjects with increased risk of PCa and might be used for clinical screening of healthy subjects. Subjects with the
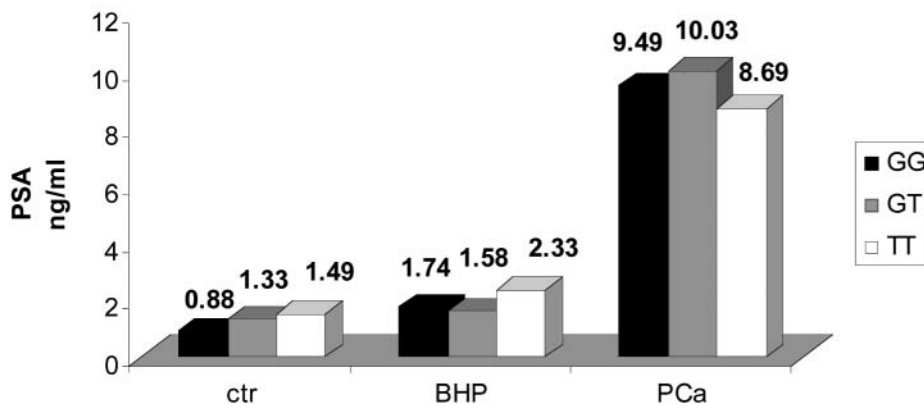
Age range 56-65 years

Panel A

Anova test in PCa patients: GG (23.48±18.63) *vs.* GT or TT genotype (9.80±6.95), F=7.021, *p*=0.014.

Age range 66-75 years

Panel B

Anova test: *p*>0.15.

Figure 1. *Plasma levels of total PSA in controls, BHP and PCa patients according to the different genotypes (GG, GT, TT) of ACT polymorphism in two different age groups.*

GG genotype or the G allele and increased PSA levels may then be followed-up for diagnostic focus and may be candidates for preventive and prospective investigations. This procedure may be of great value for prevention protocols of PCa.

Acknowledgements

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