Evidence for an association between the SRD5A2 (type II steroid 5α-reductase) locus and prostate cancer in Italian patients

K. Margiotti[a,b], F. Sangiuolo[a], A. De Luca[a], F. Froio[c], C.L. Pearce[d], V. Ricci-Barbini[e], F. Micali[f], M. Bonafè[g], C. Franceschi[g], B. Dallapiccola[h], G. Novelli[a,*] and J.K.V. Reichardt[b]

[a]*Dipartimento di Biopatologia e Diagnostica Per Immagini, Sezione di Genetica, Università "Tor Vergata", Via di Tor Vergata 135, 00133 Rome, Italy*
[b]*Institute for Genetic Medicine, Department of Biochemistry and Molecular Biology, USC Keck School of Medicine, Los Angeles, USA*
[c]*Servizio di Patologia Clinica, Ospedale Villa San Pietro, Fatebenefratelli, Rome, Italy*
[d]*Department of Preventive Medicine, Norris Comprehensive Cancer Center, USC Keck School of Medicine, Los Angeles, USA*
[e]*Divisione di Urologia, Ospedale CSS, IRCCS, San Giovanni Rotondo, Italy*
[f]*Dipartimento di Biopatologia e Diagnostica Per Immagini Sezione di Urologia, Università "Tor Vergata", Rome, Italy*
[g]*Dipartimento di Patologia Sperimentale, Università di Bologna, Bologna, Italy*
[h]*Dipartimento di Medicina Sperimentale e Patologia, Università di Roma "La Sapienza" and CSS-Mendel Institute, Rome, Italy*

Received 14 September 2000

We have investigated the contributions of three polymorphic markers in the SRD5A2 gene to prostate cancer in a group of Italian patients. We have genotyped cases and controls for a polymorphic $(TA)_n$ dinucleotide repeat and two functional substitutions, A49T and V89L, substituting respectively alanine with threonine at codon 49, and valine to leucine at codon 89. We found a substantially increased but not significant risk associated with the 49T mutation and a reduction of risk for the V89L substitution. In conclusion, we report on preliminary evidence for both increased and decreased risk associated with separate markers at this locus.

Keywords: Prostate cancer, SRD5A2 gene, prostate cancer risk, polymorphic variant

1. Introduction

Prostate cancer (PC) is one of the most common male cancers, with an incidence rising 1% per year in Europe [18]. Despite attempts at early detection, about 30% of patients have extraprostatic involvement, and 25% bone metastases at the time of diagnosis [10]. Although pharmacological and surgical treatments have improved the prognosis, PC remains one of the most common causes of cancer deaths in the general population [4]. There is convincing evidence that PC involves genetic and non-genetic factors, including race/ethnicity, family history, diet, hormonal differences, and age [10]. There are striking differences in prostate cancer incidence rates among ethnic groups, with African-American men having the highest incidence and Japanese and Chinese men the lowest [3]. These differences have been attributed in part to a diverse concentration of circulating testosterone and its metabolites [16,17]. Androgens have been implicated in the pathogenesis of PC, since they stimulate the proliferation of human PC cells in vitro and induce PC in rodents when given in large amounts [14]. Furthermore, androgen ablation is the treatment of choice for patients with locally advanced and/or metastatic disease [9,18]. Prostatic cell proliferation by androgens is primary due to dihydrotestosterone (DHT), which is obtained by conversion of testosterone by the prostatic steroid 5 alpha-reductase (SRD5A) enzyme type II encoded by the SRD5A2 gene [6,7]. In fact, finasteride, a drug used to shrink enlarged prostates, blocks the synthesis of DHT by inhibiting SRD5A activity [2,20].

*Correspondence to: Tel.: +39 06 72596078; Fax: +39 06 20427313; E-mail: Novelli@med.uniroma2.it.

Disease Markers 16 (2000) 147–150
ISSN 0278-0240 / $8.00

Involvement of SRD5A2 gene in the PC risk is supported both by epidemiological and molecular studies in distinct racial/ethnic groups [11,12,15]. These studies demonstrated that African-American and Latino men with mutations in the gene were at higher risk of prostate cancer. In addition, serum hormone levels among different racial/ethnic groups differs with respect to SRD5A2 genotype [5,19].

We report here results of an investigation into the allele distribution of three intragenic SRD5A2 variants in controls and PC cases in an Italian collection of study participants.

2. Patients and controls

Blood samples were collected from 108 patients with clinical diagnosis of PC. Patients were recruited as consecutive cases of prostate cancer in two hospitals (Casa Sollievo della Sofferenza, San Giovanni Rotondo and Ospedale Fatebenefratelli, Rome). The geographic origin of all patients was established by assessing the birthplace of grandparents. Only patients originating from Lazio (Center) and Puglia (South Italy) regions were included in the study. Each patient was accurately evaluated by histopathological, biochemical and immunological analysis. All patients were considered sporadic since they did not have an affected first degree relative. For all patients, age of onset (range 60–80 years) and Gleason score (mean 3.09 ± 1.1 SD) were available.

The 121 controls were recruited from a group of unrelated centenarians and other controls with an age distribution in the range of 70–110 years [8]. All controls were selected on the basis of regional origin (Lazio and Puglia) matching the origin of patients. No familial history of PC was documented on the basis of medical records available. They were considered free of any symptom of PC according digital rectal examination (DRE) and prostate specific antigen (PSA) level. Ambiguous results were checked by ultrasound and biopsy.

Serum AAG (5-alpha-androstane-3-alpha, 17 beta-diol-17 beta-glucuronide) levels were evaluated using a specific radio immunoassay [7], in all subjects admitted to this study as indirect measures of 5α-reductase activity. No statistically differences were detected between patients and controls (range 6.0–7.52 ng/ml).

3. Genetic analysis

About 50 to 150 ng of genomic DNA was used in polymerase chain reaction (PCR) to amplify the 3′UTR (TA)n dinucleotide repeat polymorphism [13,15]. PCR was performed using the following infrared labeled primers, H52-31 (5′-GCTGATGAAAACTGTCAAGC TGCTGA-3′), and H52-30 (5′-GCCAGCTGGCAGAA GCCAGGAGA-3′). PCR product were analyzed on an automated DNA sequencer (LI-COR DNA 4000L) by running in parallel with a molecular weight DNA marker.

Single nucleotide polymorphisms (SNPs) within the coding exon 1 region of SRD5A2 gene were detected using single-strand conformation analysis (SSCA) as previously reported [12]. Genomic DNA was amplified by PCR using two different pairs of primers amplifying the coding region of SRD5A2 exon 1. The PCR program included an initial denaturation for 5 min at 95 °C, followed by 30 cycles of denaturation for 1 min at 94 °C, extension for 1 min at 72 °C, and annealing for 1 min at 54°C/64°C for the two amplified products of 260 and 160 bp respectively. The primers used were: SRD16F (5′-GCGGCCACCGGCGAGG-3′) and H5A32R (5′-CTGTGGAAGTAATGTACGCAGAAGA-3′) for the first part, and H5A32F (5′-TTCGCGGTGCCCGCGG GGATCCTCG-3′), and SRD32BF (5′-CGGGACGAG GGCAGTGCGCTGCACT-3′) for the second part of exon 1. Amplified products were diluted $1:1$ and subjected to single-strand conformation analysis (SSCA) in a denaturing gel electrophoresis (12.5% w/v) using the GeneGel Excel System (Pharmacia Biotech). Electrophoresis was done at 15 W for 2 hrs at 10 °C.

4. Statistical analysis

Chi square tests were used to compare the genotypes among cases and controls. Odds ratios, 95% confidence intervals and p-values are presented. Where noted, Fisher's exact test was used. Logit estimators using a correction of 0.5 in cells that contain a zero were used where noted. All significance levels quoted are two-sided. Statistical analyses were performed using the SAS program (Version 6.12, Cary, NC).

5. Results

The three polymorphic markers in the SRD5A2 gene investigated in this study included a $(TA)_n$ dinucleotide repeat in the 3′ UTR, and two missense substitutions (nonsynomous SNPs), A49T (alanine at codon 49 replaced by leucine) and V89L (valine at codon 89 replaced by leucine).

Table 1
The $(TA)_n$ dinucleotide repeat, V89L polymorphism, A49T mutation in the SRD5A2 gene in Italian prostate cancer cases and controls

	$(TA)_0$ $(TA)_0$	$^*(TA)_0$ $(TA)_{8+}$ + $(TA)_9$ $(TA)_9$	**VV + VL	LL	AA	AT
Controls	75	32 (31+1)	107 (67+40)	9	112	0
Cases	84	27 (26+1)	105 (54+51)	3	103	3
Odds Ratio (OR)	0.95 (95% CI=0.51, 1.72) (p-value=0.85)		0.35 (95% CI=0.09, 1.32) (p-value=0.12)		7.7 ***(95% CI=0.39, 150.54) (p-value=0.11)	

Notes: *Non-$(TA)_0$ genotypes were combined for analysis but are reported individually in parentheses. Alleles larger than $(TA)_8$ are reported as $(TA)_{8+}$. **Non-mutant (i.e. L) genotypes were combined for analysis but are reported in parentheses. ***No TT genotypes were observed. Logit estimates use a correction of 0.5 in the cell that containe a 0. The OR calculations used Fisher's exact test (2-sided).

The number of TA repeats in PC patients and controls are shown in Table 1. Three different TA repeat alleles consisting of 0, 8 and 9 $(TA)_n$ repeat units were detected (Table 1). The predominant allele had 0 repeats in both cases and controls (Table 1). No alleles with a $(TA)_n$ repeat number exceeding 9 were detected in either of the examined groups. There was no statistically significant difference in TA repeat length among cases and controls, ($OR = 0.95$, 95% $CI = 0.51 - 1.72$). Similarly, there was no statistically significant difference in risk when a functionally relevant polymorphic variant, V89L [1,11] was examined in controls and PC patients (Table 1). However, we noted an apparent protection by the mutant L (leucine) allele of the V89L polymorphism in LL homozygotes, with an OR of 0.35 (Table 1; p-value = 0.12).

Finally, we investigated the A49T mutation which was shown to significantly increase the risk of PC in African-American and Latino men [5,12]. We detected an increased risk for carriers of the mutant T allele. However this mutation was rare and never observed in the control population (Table 1). The odds ratio of 7.7 is of a magnitude similar to the one previously reported by Makridakis et al. [12], although this finding is not statistically significant (p-value = 0.11).

An underlying concern of this study is the use of centenarians as the control population. It is not clear if they are representative of the case population. However, this selected group of healthy individuals may be considered an useful models for evaluating the impact of genetic risk factors on survival and longevity. Hence, an under representation of SRD5A2 variants involved in PC would be expected in a group of people who reach very old age in good health and who have escaped any overt cancer disease. The results are consistent with those previously published [5,12] and support an effect of the SRD5A2 gene also in the Italian patients. Interestingly, we found evidence for the presence of both susceptibility and resistance (or protection) alleles to prostate cancer at this locus in the Italian population. In particular, the A49T mutation seems to increase the risk (Table 1), while the V89L substitution appears to have a protective effect (Table 1).

6. Conclusions

Our data support the notion that the SRD5A2 locus acts in a variety of populations and it should be investigated in high and low risk populations throughout the world for its contributions to prostate cancer. Although we are confident that present results need to be corroborated by additional studies using larger samples of patients and population-based controls, they provide the first evidence for a contribution of the SRD5A2 locus to the prostate cancer risk in Italy.

Acknowldegements

We thank Dr. C.A. Asero, Dr. P. Pierogrossi, Dr. M. Pulone, Dr. C. Latiano, Dr. M. Santodirocco, Dr. G. Vespasiani, and Dr. S.M. Di Stasi for recruiting patients. Work supported by grants from NIH CA68581 (to J.K.V.R.), Italian Ministry of Health (to B.D.), and AIRC ("Healthy centenarians as a model to study genetic and cellular factors involved in cancer susceptibility", to C.F.).

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