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Short alleles of both GGN and CAG repeats at the exon-1 of the androgen receptor gene are associated to increased PSA staining and a higher Gleason score in human prostatic cancer

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ABSTRACT

The exon 1 of the human androgen receptor (AR) gene contains two length polymorphisms of CAG (polyglutamine) and GGN (polyglycine). "In vitro" experiments suggest that the larger GGN repeats provide a lower AR-protein yield, whereas the larger CAG repeats decrease the AR transcriptional activity, both decreasing the AR signalling intensity. Here we have tested such possibilities in human prostatic cancer (CaP) specimens.

We used 72 archival samples of radical prostatectomy. Parallel slides were used for AR protein or PSA immunohistochemistry, and for genotyping studies. Polymorphisms were genotyped by PCR, fragment length analysis and sequencing selected samples.

The AR staining was positively correlated with the Gleason score ($r = 0.320$; $P = 0.005$), but it was not correlated to CAG or GGN repeat length or PSA staining. The number of GGN repeats was negatively correlated to the intensity of PSA staining ($r = -0.243$; $P = 0.04$). Combination of short alleles of both tracts was significantly higher in: the heavier stained tertiles for PSA ($P = 0.03$) and AR ($P = 0.06$); and in the subgroup of samples having a Gleason score of 7 or higher ($P = 0.021$).

The results support the hypothesis that the shorter alleles of CAG and GGN repeats in the AR gene are associated to an increased AR signalling intensity in human prostate cancer, and with more aggressive forms of the disease.

1. Introduction

Prostate cancer is heterogeneous in its etiology and progression, but androgen signalling through the androgen receptor (AR) appears to be involved in all aspects of the disease [1,2]. The AR mediates the androgen action on prostatic cancer (CaP) cells at the transcriptional level, and promotes their proliferation. This AR property allows its targeting for controlling CaP growth with antiandrogens [3,4].

The AR is codified by the AR gene, which is located at the X chromosome (q11.2-q12), spans 90 kb, contains eight exons, and encodes for a protein of around 917 amino acids [5]. The N-terminal transactivation domain of the AR protein, which is indispensable for its genomic activity, is encoded by exon 1 [5,6]. This exon contains a CAG repeat (encoding for polyglutamine) highly polymorphic in length, that influences the transactivation function of AR. A lineal increase in CAG repeat length is associated with a progressive decrease in AR activity [7–9].

Several epidemiologic studies have related the CAG polymorphism is associated with the risk of developing CaP [10–16], and other steroid hormone-related tumors, such as breast, endometrial and ovarian cancer [17–20]. In a multiethnic cohort study, with a large number of prostate cancer patients, Friedman et al. did not found any association between the length of CAG repeats and prostate cancer risk [21]. The CAG tract has also been associated with some prognostic variables of CaP and even with the clinical evolution of patients [22–25]. This suggests that variation in the AR activity promoted by fluctuations in the length of the CAG repeat

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Table 1
Distribution of the androgen receptor GGN and CAG repeat alleles in a series of 72 prostate cancer and 106 sex-age matched healthy controls.

Number of repeats	GGN CaP		GGN controls (ENCA)		CAG CaP		CAG controls (ENCA)	
	n	%	*n*	%	*n*	%	*n*	%
13	0	0	1	0.9	0	0	2	1.9
14	0	0	0	0	0	0	1	0.9
15	0	0	0	0	0	0	1	0.9
16	0	0	0	0	1	1.4	0	0
17	1	1.4	0	0	1	1.4	2	1.9
18	0	0	0	0	2	2.8	7	6.6
19	1	1.4	6	5.7	8	11.1	13	12.3
20	1	1.4	3	2.8	8	11.1	13	12.3
21	0	0	4	3.8	18	25	25	23.6
22	19	26.4	12	11.3	8	11.1	7	6.6
23	44	61.1	54	50.9	7	9.7	8	7.5
24	4	5.6	19	17.9	9	12.5	12	11.3
25	2	2.8	5	4.7	3	4.2	13	12.3
26	0	0	1	0.9	1	1.4	1	0.9
27	0	0	1	0.9	2	2.8	0	0
28	0	0	0	0	3	4.2	0	0
29	0	0	0	0	1	1.4	1	0.9
Total	72	100	106	100	72	100	106	100

may affect the progression of tumors, as well as influencing their development [26].

Exon 1 of the AR gene also includes a GGN repeat (encoding for polyglycine) that is less polymorphic in length than the CAG repeat [26]. A recent report describes that the GGN repeat does not have an effect on AR transcriptional activity. Instead, a negative relation has been described between the GGN repeat length and the AR-protein yield. It has been suggested that the GGN repeats trend to form hairpins inside the AR mRNA, thus making more difficult the mRNA translation process that lead to a decreased AR-protein yield [27].

The GGN repeat seems to play a modest role in prostatic carcinogenesis [28–34]. It was initially proposed as a significant predictor of disease outcome, and since its effect was found to be stronger in early-stage tumors, it was suggested to help to identify those patients meriting more radical treatment [22]. However, the possible interest of the GGN repeat as a biological marker for CaP risk or progression is still controversial [22,35].

The AR is expressed in both stroma and epithelium of prostatic tissues and almost every CaP (over) expresses this protein [36]. The AR protein, as determined by IHC, has been used both as a prognostic factor for cancer progression and as a predictive factor for endocrine therapy [36–38]. The AR activates the expression of a large series of genes in prostate. The best studied is the kallikrein-3 (Prostate Specific Antigen, PSA), a serine protease that is synthesized and secreted both by normal and malignant epithelial cells of the human prostate. PSA is mainly induced by androgens and regulated by the AR at the transcriptional level [39]. PSA is considered an appropriate intracellular and extracellular marker to study the AR-mediated response to androgens in prostate [36].

There is no evidence in human CaP that the number of CAG repeats influence the transcriptional activity of the AR, or that the length of the GGN repeats influence the AR-protein yield. With regard to their potential clinical interest, here we have studied whether the CAG and GGN polymorphism of the AR gene are related to these AR functions and with the Gleason score in CaP specimens.

2. Materials and methods

2.1. Patients

We retrospectively studied 72 consecutive patients with CaP, diagnosed and treated at the Department of Urology of the public Hospital Universitario Insular de Gran Canaria (Canary Islands, Spain) between 1997 and 2001. All the patients underwent retropubic radical prostatectomy, including seminal vesicles, for clinically organ-confined prostate cancer. Tumors were staged using the International Union against Cancer (UICC) staging system, based on transrectal ultrasound guided biopsy and bone scans. Informed consent was obtained from every patient, and the study was approved by the hospital Committee for Clinical Investigation.

2.2. Cohort profiles

Median age 64 years (range 51–73), median preoperative serum PSA 8.1 ng/mL (range 0.2–47.7, *n* 1/4 48), median follow-up 51 months (range 36–111). Five patients scored Gleason 2–4, 18 patients scored Gleason 5–6, and 49 patients scored Gleason 7–9 by a pathologist (J.J.C.G.).

2.3. Control group

Between 1997 and 1998 a nutritional survey (Encuesta Nutricional de Canarias, ENCA) was performed on a representative sample of 1747 individuals aged 5–76 years, randomly selected from the census lists of 32 municipalities within the islands. The study was approved by the ethical committee of the Canarian Public Health Service. After giving informed consent, participants filled out a survey questionnaire with information regarding their dietetic habits, socio-demographic information as well as their personal and familial history of diabetes, hypertension, cancer and cardiovascular, hepatic and kidney disease. Current use of drugs, smoking and alcohol consumption was also recorded. Out of the 1747 individuals enrolled in the study, 782 underwent blood extractions after an overnight fast for laboratory analyses. Of these, we first excluded those individuals who could not provide any blood samples for DNA isolation procedures ($n = 58$); then, we excluded anyone younger than 18 years of age ($n = 167$). Our population study was comprised of 557 individuals (243 men and 314 women) aged 18–76 years [42–44]. Of those, 243 healthy men, the length of the AR CAG and GGN polymorphisms of 106 sex-age matched patients (range 51–73 years old) was used as a control group. No data about serum PSA were available (Table 1).

2.4. Specimen preparation

Tumor samples were fixed in neutral buffered formalin and embedded in paraffin. Blocks and sections with more than 80%

of tumors cells, were selected for sectioning and diagnosis by histopathological examination.

A 20 μm selected sections (≥80%) of tumor cells was used for DNA extraction. Each section were deparaffinized with xylene, rehydrated in an decrease ethanol concentration series and placed in 300 μL of DNA extraction buffer (NaCl 5 M, EDTA 0.5 M, Tween-20 0.5%). After proteinase K treatment, a standard phenol/chloroform and ethanol precipitation protocol was used for DNA isolation. DNA was stored at −20 °C until required.

2.5. CAG and GGN tracts analysis

To determine the length of the CAG and GGN repeats, we amplified the corresponding regions located on exon 1 of the AR gene (Genbank accession # M27423). To amplify the CAG tract, a pair of primers was designed: sense primer (cgc gaa gtg atc cag aa c) and antisense primer (aga acc atc ctc acc ctg ct). To amplify the GGN tract using a pair of primers whose sequence has been previously reported [26]. One primer from each pair was marked with fluorescent dye (FAM and VIC respectively). Amplification was performed in 25 μL reaction volume, containing 50 ng of genomic DNA, 200 μM of each deoxynucleotide triphosphate, 1× Fast Start Taq DNA polymerase Buffer (Roche Applied Science), 1× GC-rich solution buffer (Roche Applied Science) and 1 U of Fast Start Taq DNA polymerase (Roche Applied Science). The concentration of each pair of primers was 1.2 μM and 1.5 μM for the amplification of the CAG and GGN, respectively. PCR conditions were: 30 cycles of 95 °C for 45 s, 56 °C for 30 s and 72 °C for 30 s for CAG amplification; 30 cycles of 95 °C for 1 min, 55 °C for 2 min and 72 °C for 2 min for GGN amplification. Each PCR was initiated with a denaturation step at 95 °C for 5 min, and terminated with an extension step at 72 °C for 5 min. The PCR product was diluted 1:100 in distilled water and 1 μL of the dilution was mixed with 10 μL of formamide and 0.3 μL of GeneScan 500 LIZ Size Standard (Applied Biosystems), denatured at 98 °C for 5 min and cooled on ice. Fragment separation was performed by automated capillary electrophoresis, using an ABI Prism 3100 Genetic Analyzer (Applied Biosystems), and the length was determined with Gene Scan Analysis Software (version 3.7) (Applied Biosystems). Fragment size was confirmed by sequencing DNA samples harboring alleles of different sizes for both repeats by using the Big Dye Terminator Sequencing Kit (Applied Biosystem). According to the authors [40,41], the allele size estimation obtained by running the PCR products in the Gene Scan was corrected by using a ladder created with the allele size obtained by sequencing.

2.6. Immunohistochemistry

Parallel slides from paraffin blocks representative of the prostatectomy specimen were used for genotyping and immunohistochemistry (IHC) studies, as well as to establish the Gleason grade. Sections (4 μm) of the paraffin block were placed on charged polylysine-coated slides and used for morphometric hematoxilin-eosin (H&E) staining and for the IHC analyses. Antigen retrieval for IHC analyses was done in Chenmate Target Retrieval Solution Dako Cytomation for 3 min. Endogenous biotin and peroxidase were blocked using Peroxidase Blocking Dako Cytomation kit. AR protein was detected with the AR Monoclonal Antibody (Novocastra, NCL-AR-318), diluted 1:50 in Dako Antibody Diluent. Prostate Specific Antigen was detected with PSA Monoclonal Antibody (Novocastra, NCL-PSA-28 A 4) diluted 1:100. Reactions were visualized with Chenmate Dako Envision Detection Kit, Peroxidase/DAB, Rabbit/Mouse and Dab Chromogen, followed by hematoxilin counterstaining.

A single pathologist using a semi-quantitative method did the scoring for the tissue IHC. Staining intensity was evaluated in 10 randomly selected fields, and graded as absent (0), weak (1+), intermediate (2+), or strong (3+). Stained cell were also scored in the same 10 fields as follows: no staining (0), 1% to 16% of cells stained (1+), 17% to 33% of cells stained (2+), 34% to 66% of cells stained (3+), or 67% to 100% of cells stained (4+).

The IHC results are presented as: (1) staining intensity index, which represents the mean of the staining intensity of the 10 fields; (2) stained cells score, which represents the mean of the percentage of stained cells in the 10 fields; and, (3) IHC score which represents the mean of the staining intensity index and the stained cells score.

2.7. Statistics

The correlations were done using a Sperman test. Given the categorical nature of the staining factor data, contingency tables were analyzed using Pearson's χ^2 test. Mann–Whitney and Kruskal–Wallis tests were used for those analyses where continuous data was involved. All statistical analyses were done using the SPSS (version 12.0) statistical package (SPSS Inc., Chicago, IL).

3. Results

3.1. The GGN and CAG repeats in CaP

The frequency distribution of both GGN and CAG repeat alleles in our series is shown in Table 1.

In GGN repeat, the number of existing alleles was 7, the median length was 23 units (range, 13–25) and the mean was 22.6 (SD ± 1.1). Two GGN alleles, with 22 and 23 triplets, had an overall frequency of 87.5%. In CAG repeat, there were 14 different alleles, the median number of triplets was 21 (range, 16–29) and the mean was 21.9 (SD ± 2.7). Four of the CAG alleles, with 19, 20, 21 and 22 triplets, respectively, had an overall frequency of 58.3%.

The distribution frequency of the CAG and GGN alleles in the assayed samples, were compared with the general population of the Canary Islands (Table 1).

The allelic frequencies for both fragment length polymorphisms resemble those previously described for Caucasian populations, and no statistical differences were observed between cases and healthy controls. In the case of the GGN repeat, alleles larger than 24 repeats were significantly less abundant in cases than in controls (8.4% vs. 21.6%, $p = 0.01$). This interesting result needs to be confirmed in a larger CaP series.

Of the 72 assayed samples, 21 (27%) showed more than one GGN allele. The multiallelic samples contained a range of 2–5 alleles. These samples are included in the study as the mean length of detected alleles. When we considered these samples separately and made the statistical analysis, we did not find any association with the studied variables (data not shown).

3.2. AR and PSA staining in CaP

Prostatic cancer specimens selected for this work were in the 3–9 range of the Gleason score. They showed a wide variability in AR protein specific staining, often showing fields from heavily to slightly staining inside the same sample area (Fig. 1). A CaP specimen of Gleason score 8 and upper usually shows stronger AR staining at single cells on small areas. Fig. 1c is an example of this event.

The same occurred with the PSA staining that was also variable in terms of correspondence to the AR-protein staining.

In order to rationalize data for statistical analysis, we semi-quantified the AR-protein staining intensity of the strongly stained cells in each field, and also the number of stained cells with any intensity, and considered these variables either separately or in combination to obtain the IHC-score (see Section 2). Since each sample contained at least two representative tumor fields stained

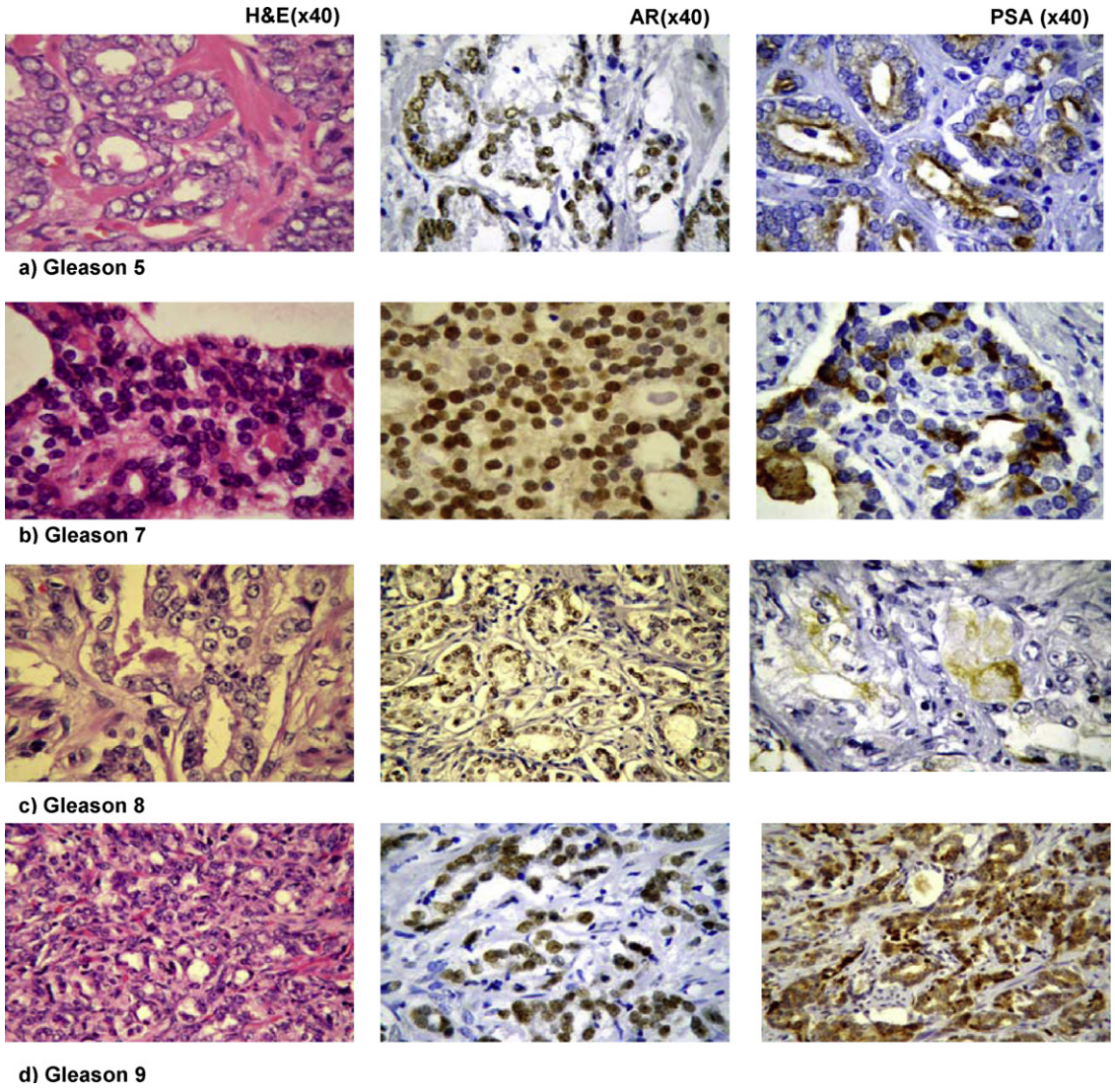

Fig. 1. Gleason Score and AR are staining or PSA staining in human prostate cancer. (a) Gleason 5 prostate carcinoma. Stained cells by HE, show the excentric nucleolus and absence of myoepithelial cells around the neoplasic glands. Immunostaining for AR show intranuclear localization and diffuse reaction with moderate intensity. Staining for PSA, show a positive reaction at the cytoplasm of the apical cells. (b) Gleason 7 prostate carcinoma. Note the compact aspect of glands. Intense and diffuse intranuclear reaction for AR antigen. PSA immunostaining show strong reaction in some cells. (c) Gleason 8, prostate carcinoma with irregular glands. In small areas, AR stain shows a moderate intensity and diffuse intranuclear expression. PSA immunoreactions show an irregular and slight stain. (d) Gleason 9. Atypical and compact disposition of glands, with infiltrating cells. AR immunostaining shows a nuclear strong signal. PSA staining with both diffuse and intense cytoplasm immunostaining.

with different intensity, we generated independent measurements of each field. Thus, we considered 170 fields out of the 72 prostates of the study. In each field we quantified three variables: staining intensity for both AR protein and PSA, and the Gleason score of each studied field.

Table 2 shows the results of data analysis for correlations between morphological variables and the CAG or GGN polymorphisms. GGN showed a negative correlation to the PSA staining.

($r = -0.232$; $p = 0.05$), but not with the AR staining. The AR staining was correlated to the Gleason score ($r = 0.320$; $p = 0.005$). There was not significant correlation of AR staining parameters with those of PSA in this series. The CAG polymorphism did not correlated with any variable.

3.3. CAG or GGN genotypes and the AR protein or PSA staining

Since this study was designed to try to find a correlation between both polymorphisms and the AR-protein yield or PSA, the CAG and GGN alleles were dichotomized using their respective median as a cut-off point, according to the strategy followed by other authors [21]. Thus, those CAG alleles with a number of triplets less than or equal to the median were considered short (S) ($n = 38$ for the S-CAG ≤ 21 repeats), and those with a number of triplets larger than the median were considered large (L) ($n = 34$ for the L-CAG > 21 repeats). GGN alleles were considered short (S) when the number of triplets was less than the median ($n = 22$ for the S-GGN < 23 repeats), and large (L) when the number of triplets was equal or higher than the median ($n = 50$ for the L-GGN ≥ 23 repeats).

Using tertiles, the PSA and AR protein were grouped according to the staining intensity. The Gleason score was also dichotomized according the clinical criteria (<7 or ≥7). Table 3a shows the results according to the Pearson's χ^2 test; in the upper tertile, tumors with short CAG alleles had higher AR intensity than those with large CAG repeats (47.4% vs. 26.5% $p = 0.052$). Tumors with short CAG repeats alleles, present a trend to have a high Gleason score (≥7), than those with L-CAG repeats alleles (76.3% vs. 58.8%, $p = 0.09$) (Table 3b).

Table 2
Spearman correlations.

$N = 72$	CAG	PSA (St. Cells)	PSA (St. Score)	Ar (St. Cells)	Ar (St. Score)	Gleason score
GGN	$r = 0.207$	−0.243	−0.232	−0.045	−0.029	0.028
	$p = 0.08$	0.04	0.05	0.71	0.806	0.813
CAG	$r =$	−0.173	−0.125	−0.199	−0.203	−0.21
	$p =$	0.145	0.296	0.094	0.087	0.077
PSA		$r =$	0.974	0.117	0.123	−0.016
(St. Cells)		$p =$	0.000	0.326	0.303	0.894
PSA			$r =$	0.130	0.143	−0.024
(St. Score)			$p =$	0.275	0.231	0.841
Ar				$r =$	0.985	0.302
(St. Cells)				$p =$	0.000	0.010
Ar					$r =$	0.320
(St. Score)					$p =$	0.005

Table 3a
CAG and GGN genotypes and the AR protein or PSA staining.

Pearson χ^2		GGN ($n = 72$)					CAG ($N = 72$)				
		<23		≥23			≤21		>21		
		n	%	n	%	p value	n	%	n	%	p value
psa intensity	0.2–1.2	8	36.4	21	42.0	0.582	15	39.5	14	41.2	0.746
	1.3–1.7	6	27.3	14	28.0		10	26.3	10	29.4	
	1.8–2.7	8	36.4	15	30.0		13	34.2	10	29.4	
psa stained cells	0.1–2.5	5	22.7	19	38.0	0.079	10	26.3	14	41.2	0.066
	2.6–3.2	6	27.3	17	34.0		11	28.9	12	35.3	
	3.3–4.0	11	50.0	14	28.0		17	44.7	8	23.5	
psa score	0.2–1.9	5	22.7	18	36.0	0.140	11	28.9	12	35.3	0.314
	2.0–2.4	7	31.8	18	36.0		12	31.6	13	38.2	
	2.5–3.3	10	45.5	14	28.0		15	39.5	9	26.5	
AR intensity	0.2–1.0	8	36.4	17	34.0	0.926	11	28.9	14	41.2	0.213
	1.1–1.6	6	27.3	15	30.0		11	28.9	10	29.4	
	1.7–3.9	8	36.4	18	36.0		16	42.1	10	29.4	
AR stained cells	0.1–3.3	6	27.3	20	40.0	0.271	10	26.3	16	47.1	0.088
	2.4–3.4	7	31.8	15	30.0		13	34.2	9	26.5	
	3.5–4.0	9	40.9	15	30.0		15	39.5	9	26.5	
AR score	0.2–1.5	6	27.3	17	34.0	0.396	9	23.7	14	41.2	0.052
	1.7–2.4	6	27.3	16	32.0		11	28.9	11	32.4	
	2.5–4.0	10	45.5	17	34.0		18	47.4	9	26.5	

3.4. Combined GGN and CAG genotypes effects on the AR protein and PSA staining, or Gleason index

Since both CAG and GGN repeats are present in the same exon 1 of the AR gene, they may influence each other's effects. We tried to find out whether the CAG and GGN alleles considered together were associated to both AR protein and PSA staining, and Gleason score. CAG and GGN genotypes were categorized as in Table 4, thus providing three haplotypes combination: SS (S-CAG and S-GGN alleles), LL (L-CAG and L-GGN); and LS (samples with S-CAG and L-GGN genotypes plus samples with L-CAG and S-GGN genotypes).

For the purpose of this work, and as in previous articles from our group, we analyzed the series as categorized in two allele combinations: SS vs. LL + LS. Table 4 shows that the SS group was associated with the more intense PSA staining ($P = 0.032$) and AR-protein staining ($P = 0.058$). The SS group was also associated with a higher Gleason score ($P = 0.021$).

4. Discussion

Three main results can be highlighted from the above presented results: (1) the positive correlation between the Gleason score and the AR staining; (2) the negative correlation of the GGN repeat with PSA staining; and (3) the association of the genotypes containing both short GGN and short CAG alleles with a higher AR protein and PSA staining, and also with a higher Gleason score of tumors.

Studies aimed to determine the value of AR expression in prostate cancer outcome after radical prostatectomy has reported a range of results. High level of AR have been found as associated with aggressive clinical-pathologic features and decreased biochemical recurrence-free survival in these CaP patients [45,46]. Associations between AR staining and Gleason score have also been reported [47], but there is no general agreement [48–50].

In our hands, the higher AR expression is seen principally in high-grade, high-stage tumors, the type of prostate carcinomas that

Table 3b
CAG and GGN genotypes and Gleason score.

Pearson χ^2		GGN ($n = 72$)					CAG ($N = 72$)				
		<23		≥23			≤21		>21		
		n	%	n	%	p value	n	%	n	%	p value
Gleason score	<7	5	22.7	18	36.0	0.202	9	23.7	14	41.2	0.091
	≥7	17	77.3	32	64.0		29	76.3	20	58.8	

Table 4
Mutual influences of the combined CAG - GGN genotype on PSA, AR or Gleason index.

$N = 72$	Combined CAG-GGN Genotypes				
	SS		LL + LS		
	Cases	%	Cases	%	
PSA (Stained cells)					
0.1–2.5	11	15.3	13	18.1	
2.6–3.2	13	18.1	10	13.9	
3.3–4.0	19	26.4	6	8.3	$p = 0.032$
AR (Stained cells)					
0.1–3.3	12	16.7	14	19.4	
2.4–3.4	14	19.4	8	11.1	
3.5–4.0	17	23.6	7	9.7	$p = 0.076$
AR (Score)					
0.1–1.5	11	15.3	12	16.7	
1.7–2.4	12	16.7	10	13.9	
2.5–4.0	20	27.8	7	9.7	$p = 0.058$
Gleason score					
<7	9	12.5	34	47.2	
≥7	14	19.4	15	20.8	$p = 0.021$

SS = CAG ≤ 21 and GGN <23 repeats; LL = CAG >21 and GGN ≥23 repeats; LS = CAG ≤ 21 and GGN ≥23 repeats or CAG >21 and GGN < 23 repeats.

fail to have a durable remission [47]. These findings suggest that an increased AR signalling is associated with a high Gleason score, suggestive of a potentially worse prognosis of prostate cancer patients treated with radical prostatectomy.

Molecular observations in prostate cell constructs support the hypothesis that short GGN repeats would lead to a lower AR-protein yield [27], but yet there is no physiological or pathological confirmation. Here we studied the association between GGN repeat length and AR-protein staining intensity, and we did not find any direct evidence supporting that hypothesis. However, the negative correlation between the length of the GGN repeat and PSA staining supports that this repeat influences the AR signalling intensity in human prostate cancer. The influence could be explained if short glycine repeats would render an increased AR transcriptional activity, a possibility that has not been previously reported. However, we cannot rule out the possibility that short GGN repeats may lead to an increased AR mRNA translation into AR protein at a level undetectable by the semi-quantitative approach used here.

Since we did not find any correlation between the CAG repeat and PSA staining, our results suggest that the influence of the GGN repeat on AR signalling could be stronger than that of the CAG repeat.

Interestingly, we found evidence that both CAG and GGN repeats influence each other's function in human prostate cancer. In fact, the association of the combined S-CAG and S-GGN on AR protein or PSA staining was found to be stronger than each repeat studied separately. The cause of a decreased translation of AR mRNA with large GGN repeats into AR protein has been attributed to hair-pin formation in the tertiary structure of the AR mRNA [27]. However, it has been described that both CAG and GGN repeats are able to form such hair-pin structures that difficult mRNA translation [51]. Therefore, it makes sense that the combined S-CAG and S-GGN genotypes are related to an increased AR signalling, despite no experimental evidence which supports this possibility.

Patients bearing both S-CAG and S-GGN repeats in this series were also associated to higher Gleason indexes. This finding is in agreement with the positive correlation between the AR staining and Gleason score found here, and also by other authors [52]. The available data on AR gene polymorphisms allow us to conclude that the combination of both short CAG and short GGN alleles intensify the AR signalling in human prostatic cancer, and are indicative of a worse prognosis of patients treated with radical prostatectomy.

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