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Androgen receptor and vitamin D receptor gene polymorphisms and prostate cancer risk

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ABSTRACT

We study the CAG repeat region in exon 1 of the androgen receptor (AR) and the *TaqI* polymorphism in exon 9 of the vitamin D receptor (VDR) and the association with prostate cancer. 137 incidentally discovered, histologically verified prostate cancers were analysed for CAG repeat length in AR and genotype at the *TaqI* site of the VDR. 124 control subjects were analysed to determine the CAG repeat length and *TaqI* genotype determined for 176 control subjects. An unpaired *t*-test shows that the mean CAG repeat length was significantly ($p < 0.001$) shorter among cases (20.1 repeats) compared with controls (22.5 repeats). Dividing the prostate cohort and controls into tertiles ($\leqslant$19, 20–22, $\geqslant$23 repeats) shows that short repeats are significantly more common among cases (odds ratio (OR) 4.45, $p = 0.00003$). Genotype frequencies for the *TaqI* polymorphism reveals no significant differences between cases and controls. We conclude that men with a short CAG repeat in the androgen receptor gene have an increased risk of developing prostate cancer.

1. Introduction

Prostate cancer represents a major health challenge being the most commonly diagnosed malignancy in men, accounting for nearly a third of cancers affecting men. In Sweden, annually, 9000 new cases are diagnosed and 2300 men die from the disease (National Board of Health and Welfare, Sweden). Over the years there has been much debate over the roles of polymorphisms in the androgen receptor (AR) and vitamin D receptor (VDR) genes in prostate cancer risk. Both have generated several studies and divided the research community into those that argue for an increase in risk associated with these polymorphisms and those that find no proof of such.

Androgens are needed for the growth and differentiation of the prostate, an effect mediated through the androgen receptor.[1] The AR gene encodes a ligand dependent transcription factor and is located to chromosome Xq11-12. In exon 1, which comprises the transactivating domain, there is a highly variable trinucleotide CAG repeat, coding for a polyglutamine chain. A shorter allele has been shown to increase transactivation activity.[2] Two possible mechanisms have been proposed; first, the triplet region may act as an inhibitor of transactivation, where longer repeats act as more effective inhibitors. Alternatively, the receptor with shorter repeat regions may have a more stable conformation.

A meta-analysis of studies conducted until February 2004 revealed a summary odds ratio (OR) of 1.19, indicating a very slight increase in risk associated with shorter CAG repeats.[3] However, the very modest increase in risk might be attributed to the fact that this study considered populations of diverse ethnicity and hence there might be populations with a low incidence of prostate cancer diluting the risk. Sweden is

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among the countries with the highest incidence of prostate cancer in the world, and since the only study conducted on a Swedish population so far[4] was on a small number of patients with sporadic prostate cancer, we sought to find whether there is an association between short CAG repeats and an increase in cancer risk.

In this study, we also investigate the *TaqI* polymorphism in the vitamin D receptor gene (VDR). Vitamin D is known to have antineoplastic, as well as antimetastatic properties, it seems likely that a defective vitamin D pathway may influence the development and progression of prostate cancer. The growth regulatory effects of the physiological active form of vitamin D, 1,25-D_3, are mediated by its nuclear receptor, the vitamin D receptor (VDR), which belongs to the nuclear receptor family of ligand-dependent transcription factors.

The *TaqI* polymorphism is located in exon 9 of the VDR gene and is in strong linkage disequilibrium with RFLPs for *Bsm*I and *Apa*I in intron 8 and a polymorphism in the 3′-UTR of the VDR gene.[5] Since the three RFLPs are in linkage disequilibrium they render two distinct haplotypes: baT and BAt (capital letters indicate absence of restriction site). The BAt haplotype has been shown to correlate with increased transcriptional activity and mRNA stability, as well as high serum levels of 1,25-D_3. Studies of these polymorphisms have so far not yielded any conclusive evidence and most studies are summarised in a meta-analysis by Ntais and colleagues.[6] The meta-analysis present results indicative of no association between the polymorphisms in the vitamin D receptor gene and prostate cancer. Since Sweden has a relatively high incidence of prostate cancer we analysed the prevalence of the *TaqI* genotype in a high risk population.

In this study we find that a short CAG repeat in the AR gene is associated with an increased risk of developing prostate cancer. The *TaqI* polymorphism in the VDR gene was not associated with increased prostate cancer risk, nor could any association be observed for the combined AR and VDR polymorphisms.

2. Materials and methods

2.1. Subjects

Tumours analysed ($n = 137$) were collected by transurethral resection at Vrinnevi hospital in Norrköping, from patients with histologically verified prostate cancer, under the time period 1987 to 1996. These patients are part of a large study and an extensive databank of these patients has been collected over the years. Data collected includes TNM staging (according to UICC 1992), tumour grading according to WHO standard, Gleason score (available for 121 patients), PSA (available for 42 patients), primary treatment, cause of death (in cases applicable) and relevant dates, such as date of birth, date of diagnosis, starting date of treatment, date of death. The age at diagnosis ranged from 61 years to 90 years, with a mean of 76.2 years and a median age of 76.5 years.

Randomly chosen blood donors from the population register have been collected to establish a DNA bank and which were used as a control population (approved by the Ethics Committee at Linköpings University, # 98110). There is DNA from 390 men in the bank of which 176 are above 60 years of age and thus suitable as a frequency matched control group. We do acknowledge the possible presence of occult prostate cancer within this group, but the frequency will be a low number and will not affect the analysis. The genotype frequencies for the VDR *TaqI* polymorphism were in Hardy Weinberg equilibrium ($\chi^2 = 2.87$ with a χ^2 limit of <3.841 at 1 degree of freedom).

2.2. Genotyping of AR CAG repeat length

A PCR fragment was generated using primers flanking the polyglutamine tract in exon 1 of the AR gene. The DNA was amplified using forward primer, AR-CAG F, 5′-ACC GAG GAG CTT TCC AGA AT-3′ and reverse primer, AR-CAG R, 5′-CTG CCT GGG GCT AGT CTC TT-3′ in a reaction volume of 20 μl containing 50 ng of DNA, 1 × PCR buffer (200 mM ammonium sulphate, 750 mM Tris pH 9.0, 0.1% Tween 20), 1.5 mM $MgCl_2$, 200 μM deoxynucleotide triphosphate, 20 pM of each primer and 0.5 units of Taq polymerase. The DNA was initially denatured at 95 °C for 5 min, thereafter cycled 35 times at 95 °C for 30 s, 62 °C for 30 s, 72 °C for 1 min, terminating the reaction with a final extension at 72 °C for 5 min. One μl of the PCR product was subjected to an additional 15 cycles where 0.5 μCi [α^{32}P]dATP was included. The samples were analysed on a denaturing 6% PAGE containing 7 M UREA. A sequenced sample with a known sequence and repeat length was run on the gel simultaneously with the samples and used as a molecular size marker. 7% ($n = 10$) of the samples were chosen at random to be analysed a second time to verify accuracy, giving a 100% concordance.

2.3. Genotyping of VDR TaqI

Codon 352 in exon 9 of the VDR gene contains a silent polymorphism, ATC or ATT, both triplets coding for isoleucine. The C → T change is associated with loss of a *TaqI* restriction site. Alleles are thus either designated t (*TaqI* site present) or T (*TaqI* site absent). VDR *TaqI* genotypes were determined by a PCR based method previously described.[7] 15% ($n = 20$) of the samples were chosen at random to be analysed a second time to verify accuracy, giving a 100% concordance.

2.4. Statistics

OR and relative risk (RR), and corresponding 95% confidence intervals (CI) were calculated as risk measurements. An unpaired *t*-test was used for comparing mean CAG repeat lengths among cases and controls.

3. Results

Prostate cancer progression does not seem to give rise to cell clones with altered repeat structure since we did not find any samples yielding two different repeat lengths. The length of the polyglutamine chain in the patient group ranges from 9 to 34 repeats, with a mean length of 20.1 repeats (SD 3.73). The control group display lengths ranging from 16 repeats to 37 repeats, with a mean length of 22.5 repeats (SD 2.85) (Table 1). Since both populations follow Gaussian distribution, we conducted an unpaired *t*-test comparing the cases and

Table 1 – CAG repeat length in exon 1 of the androgen receptor (AR)

CAG repeats[a]	Case	Control	OR	Cornfield 95% CI	P-value
⩾23	41	53	1		
20–22	54	61	1.14	0.64–2.06	0.630
⩽19	42	11	4.94	2.13–11.64	0.00003
Range	9–34	16–37			
Mean	20.1	22.5			
Median	21	22			
SD	3.730	2.850			

a CAG repeat lengths are divided in tertiles. Fewer or equal to 19 repeats is considered to be short, lengths of 20–22 repeats intermediate and ⩾23 repeats long.

controls, which shows a significant ($p < 0.001$) difference in mean repeat length between the groups. The power for detecting this difference, with a p-value of 0.001, is >99%.

In order to compare our results with previous studies we divided the study populations. Dichotomising cases and controls based on the median repeat length in the case group revealed an OR of 2.36 ($p = 0.001$). Categorising CAG repeat lengths in tertiles, with cut-offs for case population in three parts with equal number of subjects, defined short alleles as having ⩽19 repeats, intermediate alleles 20–22 repeats and long alleles have ⩾23 repeats. The prostate cancer cases displays a distribution of 42 (30.7%) subjects with short alleles, 54 (39.4%) with intermediate length alleles and 41 (29.9%) with long alleles. Of the 124 subjects analysed in the control population, 11 (8.8%) have short alleles, 61 (48.8%) have intermediate length alleles and 53 (42.4%) have long alleles. OR for intermediate and short alleles are 1.14 and 4.45 respectively, with 95% CI of 0.64–2.06 ($p = 0.630$) and 2.13–11.64 ($p = 0.00003$), indicating a significant increase in risk for individuals carrying the shorter alleles.

The association of CAG repeat length with Gleason score was analysed in the 121 patients where Gleason score was documented (Table 2). No indication of any association with Gleason score was found in this material, neither when dichotomising nor when dividing into tertiles. Furthermore, no association was observed with age of onset or cause of death.

Out of 137 patients, 51 (37.2%) revealed homozygosity for the absence of the *TaqI* restriction site in the vitamin D receptor gene, 63 (46.0%) showed a heterozygous genotype and 23 (16.8%) had two normal alleles (Table 3). This distribution is similar to that of *TaqI* genotypes in the frequency matched control group ($n = 176$), which is 67 (38.1%), 78 (44.3%) and 31 (17.6%) in the genotypes TT, Tt and tt respectively. No association between the VDR *TaqI* polymorphism and risk of prostate cancer or any of the clinical parameters. We determined the VDR genotype of the remaining men in our DNA bank (men 60 years or younger) to compare these with the frequencies previously determined. No difference was seen between the groups indicating that the control group of men above 60 years of age is representative for the Swedish male population in general.

We did not find any proof of a synergistic gene–gene effect between the studied polymorphisms in the androgen receptor and the vitamin D receptor (data not shown).

Table 2 – Androgen receptor (AR) CAG repeat length and Gleason score

CAG repeats	⩾7	<7	RR	Cornfield 95% CI	P-value
⩾23	24	12	1		
20–22	26	20	0.85	0.60–1.19	0.353
⩽19	25	14	0.96	0.69–1.34	0.817

Table 3 – Vitamin D receptor (VDR) TaqI genotype and allele frequencies for cases and control

Taq I genotype	Case	Control	OR	Cornfield 95% CI	P-value
tt	24	31	1		
Tt	62	78	1.03	0.52–2.02	0.935
TT	51	67	0.98	0.49–1.97	0.959
t	110	140	1		
T	164	212	0.98	0.70–1.38	0.925

4. Discussion

Earlier studies have proposed and found that shorter AR CAG repeats might be a risk factor for developing prostate cancer.[4,8–14] Without making any assumptions or subjective grouping of the study group, the un-paired student's t-test reveals that the mean repeat length is significantly shorter in the case population compared with the control population. In line with some previous studies[8–10,12,13] where the study population was dichotomised, often using the median to generate a cut-off point, we find in our study a significant association of short CAG repeat alleles and prostate cancer with a cut-off at <21 repeats (OR 2.36,$p = 0.001$). Since the cut-off points are not uniform between studies we tested dichotomising with cut-off points ranging from 17 to 23 repeats, which constantly resulted in a significant association for short alleles with prostate cancer risk. When the cut-off at <22, used in the meta-analysis by Zeegers and colleagues,[3] is applied on our data, it results in an OR of 1.64 with a p-value of 0.048.

Other studies[11,15] have chosen to divide the populations into tertiles, and application of such cut-offs, where case and control groups were divided into three groups of equal size, a significant OR of 4.94 ($p = 0.00003$) was obtained for short CAG repeats(⩽19 repeats) compared to long CAG repeat lengths(⩾23 repeats). These analyses supports the hypothesis that short CAG repeats are associated with increased risk of prostate cancer.

Short CAG repeats have been shown to render increased AR transactivation activity, explaining the increased efficiency to transmit growth signals.[2] However, several conflicting studies can also be found with no association between repeat length and prostate cancer risk.[15–20] The lack of uniform cut-off points across different studies ranging from ⩽17 to <23 for short repeats does not facilitate the understanding of the role of CAG repeat length in prostate cancer. In our study we have shown that we find a significant difference regardless of which cut-off point is set. Most importantly

the *t*-test also shows that there is an overall difference in mean lengths between the cases and controls, and not only when dichotomised or divided into tertiles. We acknowledge though that we have a limited sample size and further, taking into consideration that most of the samples show CAG alleles of intermediate size, and that repeats at the extremes are fairly few in numbers, these results can only give strong indications for a significant role of short CAG repeats.

When stratifying our material for Gleason score, age of onset or cause of death, we do not find any association, hence we believe that the AR CAG repeat polymorphism may not be involved in prostate cancer progression.

As pointed out by Giovannucci in 2002 one explanation of discordant results may be that many studies that find an association have used study populations collected prior to the era of PSA screening and thus contain more tumours with a high grade.[21] In our study population PSA was recorded for only 42 of 137 patients and 75 of 121 tumours are of Gleason grade 7 or higher. It seems likely that our tumours have similar characteristics to those studied by Giovannucci, Ingles and Hsing, studies that also find a positive correlation with short CAG repeats.[9,11,14]

Since the study of Taylor et al. emerged in 1996 it has been debated whether the *TaqI* polymorphism has an effect on the risk of developing prostate cancer. Extensive research on large prostate cancer groups have failed to clarify this question.[22–28] The meta-analysis conducted by Ntais and colleagues of 14 studies on the *TaqI* polymorphism show that there is no association with prostate cancer susceptibility.[6] In this study we cannot find any support for an association between the *TaqI* polymorphism and development or progression of prostate cancer. The frequencies reported for the population in the south-eastern part of Sweden are uniform with frequencies found for control populations in other studies conducted on western populations of Caucasian origin.[22–25,29] Examining the chormosomal regions in close proximity of the VDR gene might reveal haplotypes and the location of a yet unknown candidate gene or mutations in linkage disequilibrium for prostate cancer.

Conflict of interest statement

None declared.

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