# Vitamin D receptor, HER–2 polymorphisms and risk of prostate cancer in men with benign prostate hyperplasia

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## ABSTRACT

**Objectives:** Prostate cancer (PRCa) is one of the most common causes of cancer death in men and determinants of PRCa risk remain largely unidentified. Benign prostatic hyperplasia (BPH) is found in the majority of ageing men and has been associated with PRCa. Many candidate genes have been suggested to be involved in PRCa, such as those that are central to cellular growth and differentiation in the prostate gland. The vitamin D receptor (VDR) and HER-2 protooncogene have been shown to be involved in the regulation of cell proliferation and differentiation in prostate cells. Genetic variations of these genes could be useful to detect BPH patients that have a higher risk of developing PRCa. This study used a case-control design to assess the predictive value of 3 polymorphisms in VDR (*Taq*I and *Fok*I) and HER-2 (Val655Ile) to determine the risk of developing PRCa in patients with BPH.

**Methods:** Polymorphisms were detected by RFLP analysis. The study evaluated 28 patients who presented with PRCa at least 6 years after the diagnosis of BPH and 56 matched patients with BPH who did not progress to PRCa over a comparable period. The study was carried out in University of Aberdeen, Foresterhill, Aberdeen, United Kingdom in the year 2002.

**Results:** Among the case group, 89% had a TT *Taq*I genotype, whereas 57% of control had this genotype (odds ratio [OR] = 5.16, 95% confidence interval [CI] = 1.46-18.22). A similar pattern was seen for the *Fok*I genotype, although this was not statistically significant (OR = 2.33, 95% CI = 0.86-6.29). The frequency of the HER-2 Ile/Ile genotype was higher in cases (79%) compared to control subjects (66%), although this was not statistically significant (OR = 1.94, 95% CI = 0.67-5.63).

**Conclusion:** This study shows that the VDR *Taq*I polymorphism is associated with a group of men with BPH who are at an increase risk of PRCa, providing a potential tool to assist prediction strategies for this important disease.

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Prostate cancer (PRCa) constitutes a major health issue worldwide.[1,2] The etiology of PRCa is unclear, although current evidence suggests that PRCa is the result of multiple factors that include ethnicity, environmental, genetics, hormonal and dietary factors.[3-8] Benign prostatic hyperplasia (BPH) is a non-neoplastic enlargement of the prostate. It is extremely common, with a rapid increase in prevalence in the forth decade of life. According to epidemiological studies most cancers are associated with BPH elsewhere in the prostate (83.3%)[9,10] and approximately 3-20% of patients

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undergone transurethral prostatectomy (TURP) or open prostatectomy for BPH subsequently develop PRCa.[10-12] Compared to men without BPH, those with the condition have a 5-fold raised risk of developing PRCa and a 4-fold raised risk of death from PRCa.[11] A previous study reported that a family history of prostate disease (PRCa or BPH) was more frequently seen in relatives of men with BPH (20%) than in relatives of men with PRCa (12.8%) or in healthy controls (5.1%).[13] In addition, in vitro malignant transformation of BPH tissue has been previously reported.[14-16] These results suggest that common genetic mechanisms may predispose to benign and malignant prostate disease. Moreover these results suggest that BPH may be part of a premalignant environment condition in the prostate gland. With the increasing incidence of PRCa in many populations there is an urgent need for the identification of molecular markers that can serve as indicators of disease risk to focus chemoprevention and early detection strategies. Vitamin D has been implicated in PRCa, with several epidemiological studies linking low vitamin D levels with increased risk of PRCa.[17,18] Vitamin D receptor (VDR) regulates transcription of numerous genes, including genes, which are involved in the prostate cell growth and differentiation.[19] A *Taq*I single nucleotide polymorphism (SNP) in exon 9 of the VDR 3`UTR regions (C352T) has been demonstrated to effect transcriptional activity and mRNA stability, thus altering the abundance of VDR, and in turn affects vitamin D level.[20] Previous studies observed an association between the *Taq*I polymorphisms and PRCa risk.[21,22] *Fok*I is another polymorphism in VDR gene and this SNP appears to alter VDR transcriptional activity.[23] HER-2 proto-oncogene is expressed in healthy prostate and over expression has been implicated in the neoplastic transformation of prostate carcinoma.[24-26] A SNP (Val655Ile) in the transmembrane domain coding region has been identified in the HER-2 gene.[27] The Val allele has been associated with an increased risk of breast cancer, particularly among younger women.[28] This study determined if SNPs of the VDR (*Taq*I and *Fok*I) and HER-2 (Val655Ile) genes were related to risk of developing PRCa in patients with BPH.

Methods. This study took advantage to a comprehensive population-based health care system to identify a well-defined case-control study nested within a cohort with BPH. The initial data set contained 11,606 BPH biopsies representing all histologically proven cases of this disease in Northeast Scotland (Grampian region) from 1974-1990. A total of 1896 patients had >1 prostate biopsy during this period of time. Cases and control were selected from within this group. Cases were patients with only BPH in the initial biopsy and a second biopsy with PRCa obtained 6 years after the BPH biopsy. Paraffin-embedded tissue samples were obtained from 28 cases; the PRCa was diagnosed between 6 and 15 years after the initial BPH sample. Two controls were matched to each case on age and year of BPH diagnosis. Controls which had biopsy proven evidence of a benign prostate 6 years (range 6-15 years) after the initial BPH procedure. All cases and controls were Caucasian, thus guarding against the effects of population stratification. All sections were rereviewed by 2 pathologists to confirm the diagnosis. While these strict selection procedures substantially reduced the number of subjects available for evaluation, it produced a powerful data set for detection of factors that predispose patients with BPH to the development of PRCa.

Deoxyribonucleic acid was extracted from formalin fixed, paraffin-embedded tissues. The tissue sections were deparaffinized with xylene and ethanol and then DNA was isolated by proteinase K digestion.[29]

***Polymerase chain reaction assay (PCR).*** Previously described primer sets were used to amplify regions of 198 and 272bp around the *Taq*I/*Fok*I polymorphic regions[30,31] and 148bp for HER-2 SNP.[32] Genomic DNA (100-500 ng) was subjected to PCR amplification in a 25 µl reaction mixture containing 10 PCR buffer (MBI, Sunderland, UK), 1 mM $MgCl_2$ (MBI), 200 M dNTP mix (Bioline, London, UK), 10 pmol of each primer, one unit of *Taq* polymerase (Roche, Lewes, UK), and sterilized distilled water. The genomic DNA was initially denatured at 94°C for 2 minutes and thereafter subjected to 35 cycles of PCR amplification with denaturation for one minute at 94°C, annealing for 2 minutes at 60°C, extension for 2 minutes and 30 seconds at 72°C, and final extension at 72°C for 10 minutes.

***Genotype analysis.*** The PCR products were digested with the restriction endonuclease. *Taq*I (Roche, Lewes, UK) at 65°C for 5 hours, *Fok*I (New England Biolabs, UK) at 37°C for 4 hours and *BsmA*I (New England Biolabs, UK) at 55°C for 4 hours. Genotypes for the 3 SNPs were determined after separation on a 3% agarose gel. Regarding *Taq*I, individuals were scored as TT homozygous (absence of *Taq*I restriction sites), Tt heterozygotes, or tt (presence of *Taq*I restriction sites), using current literature nomenclature. The 5` UTR SNP was scored as FF homozygous (absence of *Fok*I restriction sites), Ff heterozygotes, or ff homozygous (presence of *Fok*I restriction sites). HER-2 genotype was denoted as Ile/Ile homozygous (absence of *BsmA*I restriction sites), Val/Ile heterozygotes or Val/Val homozygous (presence of *BsmA*I restriction sites).

Table 1 - Distribution of vitamin D receptor *Taq*I and *Fok*I genotype and allele frequencies in case and control populations.

| Population | Genotype frequency | | | Allele frequency | | Genotype frequency | | | Allele frequency | |
|---|---|---|---|---|---|---|---|---|---|---|
| | TT n (%) | Tt n (%) | tt n (%) | T | t | FF n (%) | Ff n (%) | ff n (%) | F | f |
| Case (n=28) | 25 (89) | 2 (7) | 1 (4) | 0.75 | 0.25 | 16 (57) | 10 (36) | 2 (7) | 0.75 | 0.25 |
| Control (n=56) | 32 (57) | 19 (34) | 5 (9) | 0.59 | 0.41 | 21 (37) | 24 (43) | 11 (20) | 0.59 | 0.41 |

***Statistical analysis.*** The distribution of *Taq*I, *Fok*I and *BsmA*I genotypes in the cases and controls were compared with that expected from the Hardy-Weinberg equation and with each other using the Chi-squared test. Conditional logistic regression methods were used in STATA (StataCorp, 1999) to compute odds ratios (OR) for PRCa risk and 95% confidence intervals (CI), associated with each genotype. EH algorithm software was used to determine the linkage disequilibrium between *Taq*I and *Fok*I SNPs.[33]

Results. ***Vitamin D receptor single nucleotide polymorphisms.*** The results of *Taq*I genotype analysis showed an overabundance of the TT genotype in BPH patients who subsequently developed PRCa ($p$=0.003; **Table 1**). *Taq*I genotype frequency in the control population was not significantly different from that expected from Hardy-Weinberg equilibrium, while *Taq*I in the case population was not in Hardy-Weinberg equilibrium. The OR for risk of developing PRCa was 5.16 (95% CI=1.46-18.22) in BPH patients having a TT genotype. A similar pattern was seen for *Fok*I, where the FF genotype was over represented in the cases compared to the controls (**Table 1**), although risk was not statistically significantly raised (OR=2.33, 95% CI=0.86-6.29). *Fok*I genotype frequency in the cases and controls showed no significant differences from that expected from Hardy-Weinberg equilibrium. *Taq*I/*Fok*I haplotypes were identified from *Taq*I and *Fok*I combined genotypes for all subjects (**Table 2)**, except for double heterozygotes (Tt/Ff). This group was not evaluated further, as it is not possible to determine the specific haplotype frequencies directly from single digest results and other direct methods are difficult to perform. The frequency of the FT haplotype (sum of combined genotypes: FF/TT, Ff/TT and FF/Tt) was significantly higher in the case population compared to controls ($p$=0.006; **Table 3**) and the OR for risk of developing PRCa was 4.38 (95% CI=1.13-16.91) in those with an FT haplotype compared to those with the other haplotype. The *Fok*I site at the 5` UTR of the VDR gene is approximately 75kb away from *Taq*I site, which is close, in terms of recombination. However, no linkage disequilibrium was detected between *Fok*I and *Taq*I in any populations studied.

***HER-2 single nucleotide polymorphism***. Genotype frequency in the case and control populations showed no significant differences from that expected from Hardy-Weinberg equilibrium. Among the case group, 79% had the Ile/Ile genotype, whereas 66% of control had this genotype. However, there was no statistical

Table 2 - Distribution of combined genotype for vitamin D receptor *Fok*I (F or f) and *Taq*I (T or t) SNPs in case and control populations.

| Population | Combined genotype % | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | ff/tt | Ff/Tt | FF/TT | ff/Tt | ff/TT | Ff/TT | FF/tt | FF/Tt | Ff/tt |
| Case (n=28) | 0 | 7 | 57 | 0 | 7 | 25 | 0 | 0 | 4 |
| Control (n=56) | 0 | 21 | 23 | 4 | 16 | 18 | 5 | 9 | 4 |

SNPs - single nucleotide polymorphisms

Table 3 - Derived vitamin D receptor TaqI/FokI haplotypes in the study population.

| Population | Ft | | Other haplotypes | |
|---|---|---|---|---|
| | n | (%) | n | (%) |
| Case (n=26) | 23 | (88.5) | 3 | (11.5) |
| Control (n=44) | 28 | (63.6) | 16 | (36.4) |

Double heterozygotes Ff/Tt were excluded from the haplotype counting as it is not possible to determine the specific haplotype frequencies directly from single digest results.
FT - sum of FF/TT, FF/Tt and Ff/TT combined genotypes

Table 4 - Distribution of genotype and allele frequencies for HER-2 SNP in case and control population.

| Population | Genotype frequency | | | Allele frequency | 95% CI |
|---|---|---|---|---|---|
| | Ile/Ile n (%) | Ile/Val n (%) | Val/Val n (%) | Ile | Val for allele Ile |
| Case (n=28) | 22 (79) | 5 (18) | 1 (3) | 0.88<br>0.12 | 0.77 - 0.94 |
| Control (n=56) | 37 (66) | 18 (32) | 1 (2) | 0.82<br>0.18 | 0.72 - 0.83 |

SNPs - single nucleotide polymorphisms,
CI - confidence interval

difference between these 2 groups ($p$=0.238; **Table 4**) and the OR for risk of developing PRCa in BPH patients having Ile/Ile genotype, compared to those with other genotypes was 1.94 (95% CI=0.67- 5.63).

Discussion. Prostate cancer is the one of the most commonly diagnosed cancers in men. Previous studies have defined a significant association between BPH and developing PRCa.[11,34,35] With the increasing incidence of BPH in the aging population, identification of risk factors for development of PRCa in BPH patient is an urgent necessity. Germline variation in genes directly involved in regulation of prostate cell proliferation and differentiation might be critically important in understanding carcinogenesis event of PRCa, as these variants might be used as a diagnostic, prevention, and prognostic markers for PRCa. If molecular markers in patients with BPH are shown to be predictors of eventual malignant transformation, then more intensive surveillance or early treatment could be offered to those carrying the markers of high-risk. In the converse situation, those patients who do not have a high risk of malignant transformation could be offered standard follow-up monitoring. Vitamin D has been shown to be involved in the regulation of cell proliferation and differentiation in prostate cells.[36,37] The action of Vitamin D is mediated through binding to its nuclear receptor (VDR). *Taq*I SNP in exon 9 of the VDR 3`UTR regions has been demonstrated to vitamin D level.[20] This study showed that the TT genotype was associated with a significant 5-fold increase in the risk of developing PRCa in patients previously diagnosed with BPH. These results are consistence with the previous finding of a significant association between *Taq*I SNP and developing PRCa in 2 separate series.[21,22] However, we found no association was observed between the *Fok*I SNP and risk of developing PRCa, consistent with the previous study of this variant on PRCa risk.[22] While the precise molecular basis for the association between the VDR *Taq*I SNP and PRCa risk is not established, these studies provide a strong case for its integration into disease prediction and chemoprevention investigations.

HER-2 proto-oncogene encodes a transmembrane glycoprotein (p185) with extensive homology to the epidermal growth factor receptor.[38] HER-2 have been found overexposed in PRCa and has been associated with poor prognosis and distant metastasis.[24,25,39,40] A SNP Val655Ile in the transmembrane domain coding region of HER-2 gene was previously found to be associated with an increased risk of breast cancer, particularly among younger women.[28] The current study represents the first evaluation of a HER-2 SNP in PRCa. Although we found a modestly raised risk associated with the Ile/Ile genotype, this was not statistically significant. Thus a role for HER-2 in PRCa risk is not precluded. Nor does it diminish the utility of evaluating newly discovered HER-2 SNPs as molecular markers for PRCa. Other studies are required to further elucidate whether HER-2 has a role in the development of PRCa.

The study shows that a constitutive VDR *Taq*I SNP is associated with a group of men with BPH that are at an increased risk of PRCa and may be a useful component of a polygenic prediction strategy for this important disease.

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