VITAMIN D RECEPTOR GENOTYPE rs731236 (TAQ1) AND BREAST CANCER PROGNOSIS

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Running Title: VDR polymorphisms and breast cancer-specific mortality

Keywords: Vitamin D, rs731236 (Taq1), VDR (calcitriol receptor), prognosis, breast cancer

Financial support: This study was funded by the State Ministry of Science, Research and Arts of Baden-Württemberg, the German Cancer Aid (project number 108250), and the Dietmar-Hopp Foundation.

Conflict of interests: The authors disclose no potential conflicts of interest

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Word count text: 1787
Word count abstract: 240
ABSTRACT

Several studies have suggested that the anti-cancerogenous effects of vitamin D might be modulated by genetic variants in the vitamin D receptor (VDR) gene. The association of VDR polymorphisms with breast cancer-specific and all-cause mortality after a breast cancer diagnosis remains, however, largely unexplored. We assessed the association of genetic variants in VDR (rs731236, rs1989969, rs2228570, 11568820) with breast cancer survival in a sample of 498 breast cancer patients with a mean age at diagnosis of 61 years from Saarland, Germany, who were followed for up to 5 years with respect to total and breast cancer-specific mortality (56 and 48 events, respectively). Adjusted hazard ratios with 95% confidence intervals (CI) were estimated by Cox regression models. We found that breast cancer patients homozygous for the rare allele of rs731236 (15% of the women in our cohort) had a tendency toward an increased risk for breast cancer-specific mortality. The hazard ratio (95% CI) adjusted for age and breast cancer stage was 2.8 (1.1-7.2) for breast cancer-specific mortality and 2.1 (0.9-4.9) for total mortality. Additional adjustment for family history of breast cancer, radical mastectomy, and body mass index changed only marginally the estimates. No association was found for rs1989969, rs2228570, and rs11568820. Our analysis suggests that VDR polymorphism rs731236 might be associated with breast cancer-specific mortality and if our findings are confirmed in future and bigger studies rs731236 might deserve consideration as a prognostic factor in clinical care of breast cancer patients.
INTRODUCTION

The hypothesis that vitamin D might have a positive effect in reducing cancer risk first emerged from ecological studies showing an association between cancer mortality and latitude, with lower mortality rates at lower latitudes (1). A possible explanation for this difference was thought to be the different exposure to ultraviolet radiation and its effect on the production of vitamin D. Laboratory studies corroborated this hypothesis by showing that 1,25-dihydroxyvitamin D [1,25(OH)\textsubscript{2}D] had an antiproliferative effect and was involved in the differentiation and apoptosis of cancer cells (2). It is speculated that the anticancerogenous effects of vitamin D are mediated through the vitamin D receptor (VDR) (3). In particular, 1,25-dihydroxyvitamin D, the active metabolite of vitamin D, binds to VDR and stimulates cell differentiation and immunological function (4). The VDR gene, located on the chromosome 12q13.1 (5), is expressed in most cancer cells (2) and presents several single nucleotide polymorphisms (SNP), which seem to be of relevance for breast cancer (6, 7). A possible mechanism explaining the involvement of genetic variants in VDR on breast cancer might be the observed association between serum concentrations of 1,25(OH)\textsubscript{2}D and VDR polymorphism. In particular, Morrison and colleagues found that serum concentrations of 1,25(OH)\textsubscript{2}D, which is thought to be involved in the differentiation and growth of breast cancer cells (2), varied in the different genotypes of VDR polymorphism rs1544410 (8).

However, very scant work investigated the association of VDR polymorphisms with breast cancer survival and no study analyzed the association with breast cancer-specific mortality. A German cohort study analyzing the association of VDR expression and survival among 82 breast cancer patients aged 54-95 years observed high VDR expression to be associated with better progression-free survival and overall survival compared to low VDR expression (9). The rs731236 polymorphism was assessed in 721 breast cancer patients below 65 years of age in the UK. A non-significant 55% increase in total mortality was observed.
among patients homozygous for the rare allele (10). An analysis conducted among 111 Swedish breast cancer patients below 37 years of age found a trend towards a higher survival among estrogen receptor-positive tamoxifen-treated patients homozygous for the rare allele of rs731236 (11).

Given these very few and partly conflicting results, we aimed to assess whether genetic variations in VDR, including rs731236, are associated with breast cancer-specific and all-cause mortality in a cohort of breast cancer patients from population-based studies in Germany.

MATERIALS AND METHODS

Study population

This analysis is based on two cancer cohort studies conducted in Saarland, Germany, the ESTHER II study and the VERDI study. Patients with primary breast cancer were recruited statewide in both studies in Saarland, a federal state of Germany, at their first diagnosis of cancer disease. The state of Saarland was chosen, inter alia, as highly efficient and reliable long-term follow-up of cancer patients is possible through the Saarland Cancer Registry (12). In the ESTHER II study more than 2,000 patients aged 50-74 years and diagnosed with various forms of cancer were recruited between January 2001 and December 2003 (13); in the VERDI study 908 patients aged ≤80 years with a first diagnosis of breast, colorectal, or gastric cancer were recruited between October 1996 and February 1998 (14).

Details of both studies have been reported elsewhere (13-15). In the VERDI study exact ascertainment of participation rates of breast cancer patients approached by their physician was possible, and participation was found to be very high (387 of 401 eligible women, 96.5%). Comparison with data from the Saarland Cancer Registry indicates high representativeness of the participants with respect to demographic and clinical
characteristics in both studies even though older patients and those with advanced stage disease were slightly underrepresented. Mortality follow-up was conducted by record linkage with information from population registries and public health authorities. For this analysis, we combined the data over 5 years of follow-up from women with breast cancer from ESTHER II (N = 369) and VERDI (N = 129) for whom DNA was available for genotyping of \( VDR \). Data from both studies were combined in order to ensure adequate statistical power. All patients gave informed consent, including permission for the use of their blood samples for analysing prognostic and genetic factors. Both studies were approved by the Ethics Committee of the Medical Faculty of the University of Heidelberg and the Medical Association of Saarland and were conducted in accordance with the declaration of Helsinki.

**SNP genotyping**

Details of SNP genotyping have been described previously (16). Briefly, DNA was extracted from blood samples collected at baseline and genotyped with Sequenom’s MassARRAY® system (Sequenom, San Diego, USA) in collaboration with the Women’s clinic of the University of Heidelberg. Of 299 duplicate samples, 289 (97%) were completely (100%) concordant on all loci and were used for the present analysis.

According to pertinent literature (6, 17), we analysed rs731236 (Taq1), rs2228570 (Fok1), rs11568820 (Cdx2), and rs1989969 (VDR-5132). For technical reasons we could not analyse rs1544410 (Bsm1) and rs7975232 (Apa1).

**Statistical analysis**

Descriptive statistics were used to show the baseline characteristics of the participants by study. Deviation from the Hardy-Weinberg equilibrium (HWE) was tested using chi square statistics. Cox regression models were performed in order to estimate hazard ratios for all-cause and breast cancer-specific mortality, with partial or full adjustment for the following
variables: age (included as continuous variable), breast cancer stage (III-IV vs I-II) according to the staging grouping system of the Union for International Cancer Control (UICC), family history of breast cancer (mother/daughter/sister), radical mastectomy (yes/no), body mass index (BMI; per unit). In all analyses the common homozygous genotype was used as reference group. The proportional hazards assumption was tested by including time dependent covariates in the model (18). All statistical analyses were conducted with SAS® version 9.2 (SAS Institute Inc., Cary, North Carolina). Statistical significance was defined by a two-sided p < 0.05.

RESULTS

Main characteristics of the study population are presented in Table 1. Our cohort of 498 breast cancer patients had a mean age of 61 years. During 5-year follow-up 56 women died. For 48 women a malignant neoplasm of the breast was indicated as the underlying cause of death (ICD-10, C50). Genotype distributions were very similar in the ESTHER II and VERDI sub-cohorts and no significant deviation from HWE was observed. In three of the analyzed SNPs of the VDR gene the heterozygous genotype was the most common genotype (prevalence ranging from 46% to 50%). The large majority (84%) of patients were diagnosed at stages I or II according to the staging system of the UICC. Only a small minority (4%) had distant metastasis (stage IV).

In the Cox proportional hazards model there was no indication for violation of the proportional hazards assumption. None of the time dependent covariates included in the model was significant. The results of the Cox proportional hazards model are shown in Table 2. A significant association between the rs731236 rare homozygous genotype and breast cancer-specific and all-cause mortality was found. Homozygous carriers of the rare allele had almost a threefold probability of death from breast cancer compared to homozygous
carriers of the common allele. The hazard ratio (HR) adjusted for age and stage was 2.8 with a 95% confidence interval (CI) of 1.1-7.2 (p for trend =0.0228). Additional adjustment for family history of breast cancer, radical mastectomy, and BMI changed the estimate only marginally (HR=3.0; CI 1.1-8.1). The association of all-cause mortality with breast cancer adjusted for age and stage was weaker than the association of breast cancer mortality and did not reach the statistical significance (HR=2.1; CI 0.9-4.9; p for trend=0.0733). No association was found between rs1989969, rs2228570, and rs11568820 and breast cancer or all-cause mortality in the partial as well as full-adjusted models. Unadjusted Kaplan Meier survival curves by rs731236 are represented in Figure 1.

Stratification of results by oestrogen and progesterone receptor status yielded similarly elevated hazard ratios for all subgroups, albeit with wide confidence intervals due to the low number of stratum cases (data not shown).

DISCUSSION

In this cohort of 498 breast cancer patients, we observed both in partially and fully adjusted models a strong association between rs731236 and breast cancer-specific mortality. These findings point to a possible relevance of rs731236 for breast cancer prognosis.

To our knowledge, no previous study has assessed the association of rs731236 with breast cancer-specific mortality. Increased total mortality has previously been reported for homozygous carriers of the rare allele from a study among breast cancer patients below 65 years of age, but the increase in risk was less pronounced and not statistically significant (10). Abbas et al. (17) found an association between rs731236 and oestrogen receptor-positive tumors among women carrying at least one copy of the rare allele. Curran and colleagues compared allele frequencies of rs731236 polymorphism between 135 breast cancer cases and 110 controls and observed a trend towards an increasing risk for breast cancer among those
homozygous for the rare allele, but no significant association (19). A study on the
association of breast cancer progression with rs731236 genotype found that women
homozygous for the common allele had a greater risk of developing lymph node
metastasis, but no association with breast cancer risk was observed (11).

Considering possible explanations for the association of rs731236 with breast cancer
mortality it is of interest to note that rs1544410, which is in strong linkage disequilibrium
(LD) with rs731236 (5, 8), has been found to be related to serum concentrations of
1,25(OH)₂D (8). Given the LD, it could be speculated that genotypes of rs731236, similarly
to rs1544410, also present different serum concentrations of 1,25(OH)₂D. In vitro studies
conducted on human malignant cell lines seem to indicate that the possible effects of
1,25(OH)₂D on cancerous cells are dose-dependent (2). In particular, experiments
conducted with physiological concentrations of 1,25(OH)₂D found that, in contrast to
previous results obtained with supraphysiological concentrations, 1,25(OH)₂D was
rather involved in cell proliferation than in growth arrest (2). It could be speculated that
while those homozygous for the common allele of rs731236 (TT genotype) have
concentrations of 1,25(OH)₂D favouring growth arrest, those homozygous for the rare
allele (CC genotype) tend to have rather concentrations favouring cell proliferation.
Unfortunately, we could not test this hypothesis because it was not possible to obtain
serum concentrations of 1,25(OH)₂D.

Another important limitation of the study is that, given the limited sample size and
number of events, confidence intervals around the estimated hazard ratios are rather wide.

Despite its limitations, our analysis points to a potential prognostic value of rs731236
among breast cancer patients. If our results are confirmed in further studies with larger sample
size and further differentiation in patient subgroups is possible, characterization of breast
cancer patients by rs731236 genotype may become a useful supplement for risk stratification
that might be of potential relevance for treatment decisions.
Disclosure of potential conflicts of interest: No potential conflicts of interest were disclosed.

Acknowledgements: We are grateful to Anne Langheinz for genotyping and data analysis.

Grant support: This study was funded by the State Ministry of Science, Research and Arts of Baden-Württemberg, the German Cancer Aid (project number 108250), and the Dietmar-Hopp Foundation.

REFERENCES


**Figure 1 Legend**

Product-limit survival estimates for breast cancer patients homozygous for the rare allele of rs731236 (CC genotype), heterozygous (TC genotype), and homozygous for the common allele (TT genotype).
Table 1 Characteristics of breast cancer patients by study

<table>
<thead>
<tr>
<th></th>
<th>ESTHER II study</th>
<th>VERDI study</th>
<th>Total sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of participants</td>
<td>369</td>
<td>129</td>
<td>498</td>
</tr>
<tr>
<td>Mean age at diagnosis</td>
<td>62</td>
<td>56</td>
<td>61</td>
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</table>

<table>
<thead>
<tr>
<th></th>
<th>N (%)</th>
<th>N (%)</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs731236 (Taq1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>131 (35.5)</td>
<td>40 (31.0)</td>
<td>171 (34.3)</td>
</tr>
<tr>
<td>TC</td>
<td>182 (49.3)</td>
<td>71 (55.0)</td>
<td>253 (50.8)</td>
</tr>
<tr>
<td>CC</td>
<td>56 (15.2)</td>
<td>18 (14.0)</td>
<td>74 (14.9)</td>
</tr>
<tr>
<td>rs2228570 (Fok1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>138 (37.4)</td>
<td>48 (37.2)</td>
<td>186 (37.4)</td>
</tr>
<tr>
<td>TC</td>
<td>172 (46.6)</td>
<td>63 (48.8)</td>
<td>235 (47.2)</td>
</tr>
<tr>
<td>TT</td>
<td>59 (16.0)</td>
<td>18 (14.0)</td>
<td>77 (15.5)</td>
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<tr>
<td>rs11568820 (Cdx2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>246 (66.7)</td>
<td>85 (65.9)</td>
<td>331 (66.5)</td>
</tr>
<tr>
<td>AG</td>
<td>116 (31.4)</td>
<td>40 (31.0)</td>
<td>156 (31.3)</td>
</tr>
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<td>AA</td>
<td>7 (1.9)</td>
<td>4 (3.1)</td>
<td>11 (2.2)</td>
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<tr>
<td>rs1989969 (VDR-5132)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>124 (33.6)</td>
<td>56 (43.4)</td>
<td>180 (36.1)</td>
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<tr>
<td>CT</td>
<td>174 (47.2)</td>
<td>55 (42.6)</td>
<td>229 (46.0)</td>
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<tr>
<td>TT</td>
<td>71 (19.2)</td>
<td>18 (14.0)</td>
<td>89 (17.9)</td>
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Tumor stage

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<td>Stage I</td>
<td>123 (34.5)</td>
<td>30 (32.3)</td>
<td>153 (34.0)</td>
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<tr>
<td>Stage II</td>
<td>174 (48.7)</td>
<td>51 (54.8)</td>
<td>225 (50.0)</td>
</tr>
<tr>
<td>Stage III</td>
<td>45 (12.6)</td>
<td>8 (8.6)</td>
<td>53 (11.8)</td>
</tr>
<tr>
<td>Stage IV</td>
<td>15 (4.2)</td>
<td>4 (4.3)</td>
<td>19 (4.2)</td>
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</table>

Receptor status

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<td>Oestrogen positive</td>
<td>241 (65.5)</td>
<td>87 (74.4)</td>
<td>328 (67.6)</td>
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<tr>
<td>Oestrogen negative</td>
<td>127 (34.5)</td>
<td>30 (25.6)</td>
<td>157 (32.4)</td>
</tr>
<tr>
<td>Progesterone positive</td>
<td>203 (55.2)</td>
<td>80 (68.4)</td>
<td>283 (58.4)</td>
</tr>
<tr>
<td>Progesterone negative</td>
<td>165 (44.8)</td>
<td>37 (31.6)</td>
<td>202 (41.6)</td>
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</table>
Table 2 Association of VDR polymorphisms with breast cancer-specific and all-cause mortality. ESTHER II and VERDI study, Saarland, Germany

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<tr>
<th>SNPID</th>
<th>Genotype</th>
<th>Persons-Years</th>
<th>Deaths (N)</th>
<th>Model 1 a) HR (CI)</th>
<th>Model 2 b) HR (CI)</th>
<th>Person-Years</th>
<th>Deaths (N)</th>
<th>Model 1 a) HR (CI)</th>
<th>Model 2 b) HR (CI)</th>
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</thead>
<tbody>
<tr>
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<td>TT</td>
<td>598</td>
<td>10</td>
<td>Reference</td>
<td>Reference</td>
<td>586</td>
<td>15</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td></td>
<td>TC</td>
<td>907</td>
<td>27</td>
<td>2.1 (0.9-4.7)</td>
<td>2.0 (0.9-4.9)</td>
<td>903</td>
<td>29</td>
<td>1.5 (0.7-3.1)</td>
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</tr>
<tr>
<td></td>
<td>CC</td>
<td>254</td>
<td>11</td>
<td>2.8 (1.1-7.2)</td>
<td>3.0 (1.1-8.1)</td>
<td>253</td>
<td>12</td>
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<tr>
<td>rs1989969 (VDR-5132)</td>
<td>CC</td>
<td>648</td>
<td>17</td>
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<td>Reference</td>
<td>648</td>
<td>17</td>
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<tr>
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<td>788</td>
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<td>655</td>
<td>14</td>
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<td>Reference</td>
<td>652</td>
<td>17</td>
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<td></td>
<td>TC</td>
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<td>27</td>
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<td>1168</td>
<td>34</td>
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<tr>
<td></td>
<td>AG</td>
<td>538</td>
<td>16</td>
<td>1.6 (0.8-3.1)</td>
<td>1.7 (0.8-3.3)</td>
<td>535</td>
<td>19</td>
<td>1.5 (0.8-2.8)</td>
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<td>1.8 (0.4-7.6)</td>
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<td>40</td>
<td>3</td>
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<td>1.2 (0.3-5.1)</td>
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a) adjusted for age (linear) and breast cancer stage (III-IV vs I-II),
b) additionally adjusted for family history of breast cancer (mother/daughter/sister); BMI (per unit), and radical mastectomy (yes/no)
Figure 1 Kaplan Meier plots for survival by rs731236

CC genotype
TC genotype
TT genotype
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Cancer Epidemiol Biomarkers Prev  Published OnlineFirst January 8, 2013.

Updated version
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doi:10.1158/1055-9965.EPI-12-0970-T

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