Abstract
Several studies have suggested that the anticancerogenous 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, and rs11568820) with breast cancer survival in a sample of 498 patients with breast cancer 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 HRs with 95% confidence intervals (CI) were estimated by Cox regression models. We found that patients with breast cancer 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 HR (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 only marginally changed 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 bigger studies, rs731236 might deserve consideration as a prognostic factor in clinical care of patients with breast cancer. Cancer Epidemiol Biomarkers Prev; 22(3): 437–42. ©2012 AACR.

Introduction

The hypothesis that vitamin D might have a positive effect in reducing cancer risk first emerged from ecologic 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 UV 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)2D] 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; ref. 3). In particular, 1,25(OH)2D, the active metabolite of vitamin D, binds to VDR and stimulates cell differentiation and immunologic 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 to 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)2D and VDR polymorphism. In particular, Morrison and colleagues found that serum concentrations of 1,25(OH)2D, which is thought to be involved in the differentiation and growth of breast cancer cells (2), varied in the different genotypes of VDR polymorphism rs1544110 (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 patients with breast cancer, ages 54 to 95 years, observed high VDR expression to be associated with better progression-free survival and overall survival compared with low VDR expression (9). The rs731236 polymorphism was assessed in 721 patients with breast cancer below 65 years of age in the United Kingdom. A nonsignificant 55% increase in total mortality was observed among patients homozygous for the rare allele (10). An analysis conducted among 111 patients with Swedish breast cancer below 37 years of age found a trend toward
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 patients with breast cancer from population-based studies in Germany.

**Materials and Methods**

**Study population**

This analysis is based on 2 cancer cohort studies conducted in Saarland, Germany, the ESTHER II study and the VERDI study. Patients with primary breast cancer were recruited state-wide in both studies in Saarland, a federal state of Germany, at their first diagnosis of cancer. The state of Saarland was chosen, *inter alia*, as highly efficient, and reliable long-term follow-up of patients with cancer is possible through the Saarland Cancer Registry (12). In the ESTHER II study more than 2,000 patients of ages 50 to 74 years and diagnosed with various forms of cancer were recruited between January 2001 and December 2003 (13); in the VERDI study 908 patients of ages 80 years or less 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 patients with breast cancer approached that of 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 analyzed rs731236 (Taq1), rs2228570 (Fok1), rs11568820 (Cdx2), and rs1989969 (VDR-5132). For technical reasons we could not analyze 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 χ² statistics. Cox regression models were conducted to estimate HRs 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.). 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 patients with breast cancer 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 subcohorts and no significant deviation from HWE was observed. In 3 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 3-fold probability of death from breast cancer as compared with homozygous carriers of the common allele. The HR adjusted for age and stage was 2.8 with a 95% confidence interval (CI) of 1.1–7.2 (*P* trend = 0.0228). Additional adjustment for family history of breast cancer, radical mastectomy, and BMI changed the estimate only marginally (HR, 3.0; 95% 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; 95% CI, 0.9–4.9; \( P_{\text{trend}} = 0.0733 \)). 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 Fig. 1.

Stratification of results by estrogen and progesterone receptor status yielded similarly elevated HRs for all subgroups, albeit with wide CIs due to the low number of stratum cases (data not shown).

Discussion

In this cohort of 498 patients with breast cancer, 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 patients with breast cancer below 65 years of age, but the increase in risk was less pronounced and not statistically significant (10). Abbas and colleagues (17) found an association between rs731236 and estrogen receptor–positive tumors among women carrying at least 1 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 toward 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 with rs731236 (5, 8), has been found to be related to serum concentrations of 1,25(OH)\(_2\)D (8). Given the linkage disequilibrium, it could be speculated that genotypes of rs731236, similarly to rs1544410, also present different
Table 2. Association of VDR polymorphisms with breast cancer-specific and all-cause mortality

<table>
<thead>
<tr>
<th>SNP ID</th>
<th>Genotype</th>
<th>Person-Years</th>
<th>Deaths (N)</th>
<th>Model 1 HR (95% CI)</th>
<th>Model 2b HR (95% CI)</th>
<th>Person-years</th>
<th>Deaths (N)</th>
<th>Model 1 HR (95% CI)</th>
<th>Model 2b HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs731236 (Taq1)</td>
<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>2.1 (0.9–4.9)</td>
<td>2.1 (0.9–4.9)</td>
</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>
<td>2.1 (0.9–4.9)</td>
<td>2.1 (0.9–4.9)</td>
</tr>
<tr>
<td>rs1989969 (VDR-5132)</td>
<td>CC</td>
<td>648</td>
<td>17</td>
<td>Reference</td>
<td>Reference</td>
<td>648</td>
<td>17</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>800</td>
<td>24</td>
<td>1.3 (0.3–2.7)</td>
<td>0.8 (0.3–2.0)</td>
<td>798</td>
<td>17</td>
<td>0.8 (0.3–2.0)</td>
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</tr>
<tr>
<td></td>
<td>TT</td>
<td>312</td>
<td>7</td>
<td>0.8 (0.3–2.0)</td>
<td>0.8 (0.3–2.0)</td>
<td>311</td>
<td>8</td>
<td>0.8 (0.3–2.0)</td>
<td>0.8 (0.3–2.0)</td>
</tr>
<tr>
<td>rs2228570 (Fok1)</td>
<td>CC</td>
<td>655</td>
<td>14</td>
<td>Reference</td>
<td>Reference</td>
<td>652</td>
<td>17</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td></td>
<td>TC</td>
<td>814</td>
<td>27</td>
<td>1.4 (0.7–2.8)</td>
<td>1.2 (0.5–2.3)</td>
<td>810</td>
<td>29</td>
<td>1.2 (0.5–2.3)</td>
<td>1.2 (0.5–2.3)</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>290</td>
<td>7</td>
<td>0.7 (0.2–2.0)</td>
<td>0.7 (0.2–2.0)</td>
<td>280</td>
<td>10</td>
<td>0.7 (0.2–2.0)</td>
<td>0.7 (0.2–2.0)</td>
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<tr>
<td>rs11568820 (Cdx2)</td>
<td>CG</td>
<td>1,181</td>
<td>34</td>
<td>Reference</td>
<td>Reference</td>
<td>1,168</td>
<td>34</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td></td>
<td>AG</td>
<td>538</td>
<td>16</td>
<td>1.6 (0.6–4.3)</td>
<td>1.7 (0.6–5.3)</td>
<td>535</td>
<td>19</td>
<td>1.7 (0.6–5.3)</td>
<td>1.7 (0.6–5.3)</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>40</td>
<td>3</td>
<td>1.8 (0.4–7.8)</td>
<td>1.5 (0.3–6.6)</td>
<td>40</td>
<td>3</td>
<td>1.5 (0.3–6.6)</td>
<td>1.5 (0.3–6.6)</td>
</tr>
</tbody>
</table>

NOTE: ESTHER II and VERDI study, Saarland, Germany

*Adjusted for age (linear) and breast cancer stage (III-IV vs. I-II).

**Additionally adjusted for family history of breast cancer (mother/daughter/sister), BMI (per unit), and radical mastectomy (yes/no).
physiologic concentrations, 1,25(OH)2D was rather that, in contrast to previous results obtained with supra-
out with physiologic concentrations of 1,25(OH)2D found
are dose-dependent (2). In particular, experiments carried
with physiologic concentrations of 1,25(OH)2D found
that, in contrast to previous results obtained with supra-
physiologic 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 concentra-
tions of 1,25(OH)2D favoring growth arrest, those homo-
zygous for the rare allele (CC genotype) tend to have
concentrations of 1,25(OH)2D favoring growth arrest, those homo-
zgyous for the rare allele (CC genotype) tend to have
rather concentrations favoring cell proliferation. Unfortu-
nately, 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, CIs around
the estimated HRs are rather wide.

Despite its limitations, our analysis points to a potential
prognostic value of rs731236 among patients with breast
cancer. If our results are confirmed in further studies with
larger sample size and further differentiation in patient
subgroups is possible, characterization of patients with
breast cancer by rs731236 genotype may become a useful
supplement for risk stratification that might be of poten-
tial relevance for treatment decisions.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Vitamin D Receptor Genotype rs731236 (Taq1) and Breast Cancer Prognosis

Laura Perna, Katja Butterbach, Ulrike Haug, et al.