Vitamin D receptor polymorphisms (FokI, BsmI) and breast cancer risk: association replication in two case–control studies within French Canadian population

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Abstract

Vitamin D has been associated with reduced breast cancer risk. We studied the association of two vitamin D receptor (VDR) gene single nucleotide polymorphisms restriction enzyme detecting SNP of VDR (FokI and BsmI) with breast cancer risk in two independent case–control studies carried out in the same population. The modifying effect of family history of breast cancer on this relationship was also evaluated. The first and second studies included respectively 718 (255 cases/463 controls) and 1596 (622 cases/974 controls) women recruited in Quebec City, Canada. FokI and BsmI genotypes were assessed. Relative risks of breast cancer were estimated by multivariate logistic regression. Compared with homozygotes for the common F allele (FF genotype), FokI ff homozygotes had a higher breast cancer risk (study 1: odds ratio (OR) = 1.22, 95% confidence interval (CI) = 0.76–1.95; study 2: OR = 1.44, 95% CI = 1.05–1.99; and combined studies: OR = 1.33, 95% CI = 1.03–1.73). Significant interactions were observed between FokI and family history of breast cancer in the two studies as well as in the combined analysis (P interaction = 0.031, 0.050 and 0.0059 respectively). Among women without family history, odds ratios were 1.00, 1.27 (95% CI = 1.02–1.58) and 1.57 (95% CI = 1.18–2.10) respectively for FF, Ff and ff carriers (P trend = 0.0013). BsmI Bb + bb genotypes were associated with a weak non-significant increased risk in the two studies (combined OR = 1.22, 95% CI = 0.95–1.57) without interaction with family history. Results support the idea that vitamin D, through its signalling pathway, can affect breast cancer risk. They also suggest that variability in observed associations between VDR FokI and breast cancer from different studies may partly be explained by the proportion of study subjects with a family history of breast cancer.

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Introduction


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circulating 25-hydroxyvitamin D (25(OH)D) and breast density (Brisson et al. 2007). A recent randomized controlled trial involving vitamin D supplementation sufficient to raise serum 25(OH)D > 80 nmol/l in postmenopausal women reported a decrease in all-cancer risk including a decrease in risk of breast cancer (Lappe et al. 2007).

In vivo and in vitro experimental studies have shown that vitamin D inhibits cellular proliferation, induces apoptosis and suppresses tumour growth (reviewed in Banerjee & Chatterjee (2003) and Welsh (2004)). These effects of vitamin D on cells are thought to be mediated through the vitamin D receptor (VDR) that belongs to the steroid receptor superfamily. The VDR is expressed in different tissues including normal and cancerous mammary epithelial cells (Wietzke et al. 2005). Several recent studies with transgenic mice with inactivated VDR (VDR KO mice) suggest that functional VDR tends to reduce the amount of breast tissue structures targeted in breast carcinogenesis. It was shown that VDR KO mice had heavier mammary glands, increased branching, greater ductal extension and higher number of undifferentiated terminal-end bud structures during puberty compared with the wild-type mice (Zinser et al. 2002). Moreover, knocking-out VDR induces early alveolar development during pregnancy and delays apoptotic regression during involution (Zinser & Welsh 2004b). A dose–response relationship of the VDR gene was demonstrated by crossing VDR KO mice with transgenic mice carrying the activated neu oncogene under the control of the mouse mammary tumour virus (MMTV-neu mice) where one copy of the less efficient VDR was sufficient to shorten the latency period and increase the incidence of mammary tumour formation (Zinser & Welsh 2004a).

The VDR harbours several known functional polymorphisms. Some of these polymorphisms have been analysed for potential associations with breast cancer, including the single nucleotide polymorphisms (SNPs) restriction enzyme detecting SNP of VDR (FokI; rs10735810 and BsmI; rs1544410). Being located at each end of the gene encoding VDR, they are less likely to be in linkage disequilibrium (LD) and are therefore good candidates for independent effects on invasive breast cancer risk (reviewed in Uitterlinden et al. (2004)).

Studies of VDR FokI and BsmI polymorphisms have been conducted in different populations with mixed results. Additional data are needed to clarify whether these SNPs are related to breast cancer risk. Consequently, we re-evaluated the associations of FokI and BsmI polymorphisms with breast cancer risk using two independent case–control studies carried out within the same homogeneous Quebec population.

Materials and methods

Study 1

This case–control study has been previously described (Demers et al. 2000, 2002). Briefly, from October 1994 to March 1997, 255 women with histologically confirmed primary invasive breast carcinomas were recruited in four hospitals of the Quebec City area (Quebec, Canada). Hospital and population controls, free of breast cancer, were also recruited for a total of 463. Like cases, controls had to be free of any other cancer (except cervical intraepithelial neoplasm or basocellular skin cancer) and, in addition, hospital controls had to be free of any gynaecological illnesses. Since results were essentially the same using either hospital or population controls, the control groups were combined for subsequent analyses. Cases and controls were matched for age and region of residence and were almost exclusively French Canadian. Blood samples were obtained from cases and hospital controls after surgery, and for cases prior to the initiation of chemotherapy or radiotherapy. For population controls, a research nurse collected blood samples during a home visit. Information on demographic and anthropometric characteristics, lifestyle habits and reproductive history was obtained from cases and controls by telephonic interview.

VDR genotypes were assessed as follows. DNA was extracted from whole blood with the Qiagen 96-wells procedure and reagents (Qiagen Inc). The VDR locus was genotyped for BsmI and FokI polymorphisms after PCR amplification and digestion. Primer sequences are available upon request and PCR conditions for BsmI have already been published (Giguere et al. 2000). The absence of the polymorphic site (B) resulted in an 850 bp fragment while the presence of the polymorphic site (b) resulted in 700 and 150 bp fragments (Morrison et al. 1994). For the FokI polymorphism, PCR amplification and digestion were done as published (Gross et al. 1996) except that overnight digestion with 1U of FokI was used to minimize partial digests. The absence of the FokI polymorphic site (F) resulted in a 265 bp fragment and its presence (f) in 196 and 69 bp fragments (Gross et al. 1996). The interpretation of VDR FokI and BsmI genotypes was performed without the knowledge of the status (case or control) of study subjects; only samples for which genotypes had been assigned with a 100% concordance between three independent readers were included. In case of
non-agreement, PCR amplification, digestion and genotyping were reassessed independently again. Because of limitations in the amount of DNA available, lack of concordance and some laboratory missing values, genotypes of 679 and 688 participants were obtained for FokI and BsmI respectively. We first analysed our data with hospital and population controls separately and, as results were essentially the same, control groups were merged for subsequent analysis.

Study 2

In this case–control study, 622 women with histologically confirmed primary invasive breast carcinomas were recruited from a Quebec City Breast Centre (Centre des Maladies du Sein Deschênes-Fabia) from September 1999 to November 2001. They were matched for age and region of residence with 974 controls recruited through voluntary response to local newspaper advertisement and through a preventive campaign on cardiovascular disease and osteoporosis risk factors in women, held in different public or working places. Controls had to be free of breast cancer or any other cancer (except cervical intraepithelial neoplasm or basocellular skin cancer). Details from this cohort studied for genetic and environmental risk factors affecting bone mineral density have already been described elsewhere (Blanchet et al. 2003, Laflamme et al. 2005). A total of 622 cases and 974 controls were genotyped in the same laboratory as in study 1. Participants were almost exclusively French Canadian also residing in the Quebec City metropolitan area. Blood samples, information on demographic and anthropometric characteristics, lifestyle habits and reproductive history were obtained from cases by a research nurse during a follow-up visit after breast surgery and prior to the initiation of chemotherapy or radiotherapy. For controls, blood samples and the above information were collected at recruitment.

DNA was prepared in 96-well plates from 200 μl of blood using the QIAamp blood kit (Qiagen) following the manufacturer’s recommendations. The collected DNA was further diluted five times with a solution 10 μM TRIS–HCl (pH 7.5), containing 33.7 μM of an inert fluorescent dye, 6-carboxyl-X-rhodamine (ROX) (Molecular Probes, Invitrogen). All SNPs were analysed by allele-specific PCR, primer sequences are obtained for FokI and BsmI respectively. We first analysed our data with hospital and population controls separately and, as results were essentially the same, control groups were merged for subsequent analysis.

Statistical analysis

Unconditional logistic regression was used to estimate relative risk (odds ratio) of breast cancer in relation to genotypes. Before combining the two studies, heterogeneity was examined using the likelihood ratio test to assess the statistical significance of an interaction term between study and polymorphisms. Matching variables and those known to be breast cancer risk factors were considered potential confounders and were included in the models. No attempt to reach more parsimonious models were tried in order to compare our results with the recent and largest study done to date on these SNPs (Chen et al. 2005). Effect modification (departure from odds-ratio multiplicativity) was examined by introducing an interaction term between the SNP and the risk factor under consideration in the logistic regression models with both factors as main effect, and tested as above. The modifying effects were first assessed in study 1 and those observed in study 1 were then retested in study 2. Only results for effect modification by family history of breast cancer are reported since it is the only factor showing effect modification in the two studies. In order to explore possible associations further, dose–response trend in relative risk with increasing number of risk alleles was assessed by treating the genotypes as a continuous variable (scored 0, 1 and 2) in the models. Such analyses were conducted among family history subgroups, the β coefficients representing the per-allele increase in relative risk. All statistical tests were two sided and a nominal P value of 0.05 was considered statistically significant. Analyses were performed with SAS version 8.2. Hardy–Weinberg equilibrium and the absence of LD were evaluated using the allele procedure of SAS version 9.1.
Results

The principal characteristics of women in our studies are presented in Table 1. Some differences can be found between the two studies but those differences are relatively small. For instance, women from the second study were slightly older and somewhat less likely to have a high parity, to be current smokers or postmenopausal.

Associations of FokI and BsmI with invasive breast cancer

There was no statistically significant deviation from the expected Hardy–Weinberg equilibrium in control samples for both polymorphisms in both studies. We, like others (Hayes et al. 2005, Sweeney et al. 2006), found no LD between these two polymorphisms (study 1, \( D' = 0.13 \); study 2, \( D' = 0.020 \)). Minor allele frequencies for both SNPs were similar in both studies (allele frequencies in controls: FokI: study 1, \( f = 0.38 \); study 2, \( f = 0.39 \) and BsmI: study 1, \( B = 0.42 \); study 2, \( B = 0.39 \) and genotypes frequencies in these samples from French Canadian population were similar to other Caucasian populations (Uitterlinden et al. 2004).

FokI was associated with an increased relative risk of breast cancer in the two studies reaching statistical significance in study 2 (Table 2). There was no heterogeneity between the two studies (\( P \) heterogeneity = 0.17). In the combined analysis, relative risk was 1.33 (95% CI = 1.03–1.73) for ff genotype and 1.15 (95% CI = 0.95–1.40) for Ff heterozygotes compared with those carrying the common FF genotype.

Effect modification by family history of breast cancer

When stratifying women by family history of breast cancer in first-degree relatives, a higher risk associated with FokI f allele was seen only in women reporting no family history (Table 3). Interaction between family history of breast cancer and FokI was statistically significant in both studies as well as in the combined analysis (\( P \) interaction = 0.031, 0.050 and 0.0059 respectively). Among women without family history, relative risks were 1.27 (95% CI = 1.02–1.58) and 1.57 (95% CI = 1.18–2.10) respectively for FokI Ff and ff genotypes compared with women with FF genotype. The per-allele increase in relative risk is 1.26, i.e. each additional f multiplied the relative risk by 1.26 (\( \beta = 0.2285, \ P_{\text{trend}} = 0.0013 \)). By contrast, among women with a history of breast cancer, odds ratios were slightly lower for those with f alleles (OR = 0.73, 95% CI = 0.46–1.17 for heterozygotes; OR = 0.66, 95% CI = 0.36–1.19 for homozygotes in the combined study) but neither genotype nor trend (\( P_{\text{trend}} = 0.13 \))

Bsni showed little association with breast cancer risk (Table 2) but results were similar in both studies (\( P \) heterogeneity = 0.80) and data were combined. Odds ratios for women who had one or two b allele were identical (OR = 1.21; 1.23, for Bb and bb genotypes respectively). Compared with women with the BB genotype, the combination of Bb and bb carriers suggests an association with breast cancer risk (OR = 1.22, 95% CI = 0.95–1.57, \( P = 0.12 \); data not shown).

Table 1 Characteristics of women by study

<table>
<thead>
<tr>
<th></th>
<th>Study 1 (n=718)</th>
<th>Study 2 (n=1596)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years; mean±s.d.)</td>
<td>52.3±9.7</td>
<td>55.8±10.5</td>
</tr>
<tr>
<td>Age (years) at menarche (mean±s.d.)</td>
<td>12.7±1.7</td>
<td>12.8±1.6</td>
</tr>
<tr>
<td>Family history of breast cancer (%)</td>
<td>16.0</td>
<td>16.2</td>
</tr>
<tr>
<td>Body mass index (kg/m²; mean±s.d.)</td>
<td>25.0±4.5</td>
<td>25.6±4.8</td>
</tr>
<tr>
<td>Age (years) at first birth (mean±s.d.)</td>
<td>24.8±4.3</td>
<td>25.6±4.6</td>
</tr>
<tr>
<td>Parity (%)</td>
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<tr>
<td>Nulliparous</td>
<td>18.5</td>
<td>20.2</td>
</tr>
<tr>
<td>1–2 birth</td>
<td>43.9</td>
<td>48.3</td>
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<tr>
<td>3+ birth</td>
<td>37.6</td>
<td>31.5</td>
</tr>
<tr>
<td>Smoking status (%)</td>
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<td></td>
</tr>
<tr>
<td>Non-smoker</td>
<td>49.7</td>
<td>56.4</td>
</tr>
<tr>
<td>Former smoker</td>
<td>29.2</td>
<td>27.7</td>
</tr>
<tr>
<td>Current smoker</td>
<td>21.1</td>
<td>15.9</td>
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<tr>
<td>Postmenopausal (%)</td>
<td>66.8</td>
<td>61.1</td>
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</tbody>
</table>

\( ^a \)Two missing values in study 2 (n=1594).

\( ^b \)In mother, sister, daughter; 13 missing values in study 1 (n=705).

\( ^c \)In parous women (study 1, n=585; study 2, n=1271).

\( ^d \)Three missing values in study 2 (n=1593).

\( ^e \)Six missing values in study 2 (n=1590).
reached statistical significance. No effect modification by family history of breast cancer was apparent for the relationship of $BsmI$ to breast cancer ($P_{interaction} = 0.93$; data not shown).

**Discussion**

Our results suggest that, within this almost exclusively Caucasian population of French origin, $FokI$ VDR polymorphism is associated with an increased risk of invasive breast cancer. The association of $FokI$ is stronger in women without a family history of breast cancer in first-degree relatives. $BsmI$ polymorphism appears to bear only a weak relationship to breast cancer risk, if any, in this population.

**FokI and breast cancer**

Seven papers have analysed the association of $FokI$ with breast cancer risk (Curran et al. 1999, Ingles et al. 2000, Breherton-Watt et al. 2001, Guy et al. 2003, 2004, Chen et al. 2005, McCullough et al. 2007), three of which are apparently emerging from the same UK Caucasian population sample (Breherton-Watt et al. 2001, Guy et al. 2003, 2004) and are reporting non-significant but generally lower relative risk of breast cancer for the $f$ allele carriers. Three other studies (Curran et al. 1999, Ingles et al. 2000, McCullough et al. 2007) did not observe any association. In the Nurses’ Health Study (Chen et al. 2005) conducted within US population, the $ff$ carriers showed a statistically significant increase in relative risk of breast cancer compared with women with the $FF$ genotype ($OR = 1.34; 95\% CI = 1.06–1.69$), which is consistent with our present findings ($OR = 1.33; 95\% CI = 1.03–1.73$). Moreover, both studies suggest that relative risk of breast cancer increases with the increasing dosage of the $f$ allele. If our observed associations are valid and causal, about 13 and 25% of

### Table 2
Restriction enzyme detecting SNP of VDR ($FokI$ and $BsmI$) vitamin D receptor (VDR) polymorphisms and breast cancer risk

<table>
<thead>
<tr>
<th></th>
<th>Cases $N$ (%)</th>
<th>Controls $N$ (%)</th>
<th>Adjusted OR $^a$</th>
<th>95% CI</th>
<th>$P^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FokI</strong></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Study 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>FF</td>
<td>92 (37.9)</td>
<td>164 (38.8)</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ff</td>
<td>105 (43.2)</td>
<td>196 (46.3)</td>
<td>0.96</td>
<td>0.67–1.37</td>
<td>0.83</td>
</tr>
<tr>
<td>ff</td>
<td>46 (18.9)</td>
<td>63 (14.9)</td>
<td>1.22</td>
<td>0.76–1.95</td>
<td>0.42</td>
</tr>
<tr>
<td>Total</td>
<td>243 (100)</td>
<td>423 (100)</td>
<td></td>
<td></td>
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<tr>
<td>Study 2</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>FF</td>
<td>198 (32.1)</td>
<td>353 (36.8)</td>
<td>1.0</td>
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</tr>
<tr>
<td>Ff</td>
<td>306 (49.7)</td>
<td>451 (47.1)</td>
<td>1.30</td>
<td>1.02–1.65</td>
<td>0.036</td>
</tr>
<tr>
<td>ff</td>
<td>112 (18.2)</td>
<td>154 (16.1)</td>
<td>1.44</td>
<td>1.05–1.99</td>
<td>0.024</td>
</tr>
<tr>
<td>Total</td>
<td>616 (100)</td>
<td>958 (100)</td>
<td></td>
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<tr>
<td>Combined</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FF</td>
<td>290 (33.8)</td>
<td>517 (37.4)</td>
<td>1.0</td>
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<tr>
<td>Ff</td>
<td>411 (47.8)</td>
<td>647 (46.8)</td>
<td>1.15</td>
<td>0.95–1.40</td>
<td>0.16</td>
</tr>
<tr>
<td>ff</td>
<td>158 (18.4)</td>
<td>217 (15.7)</td>
<td>1.33</td>
<td>1.03–1.73</td>
<td>0.029</td>
</tr>
<tr>
<td>Total</td>
<td>859 (100)</td>
<td>1381 (100)</td>
<td></td>
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<tr>
<td><strong>BsmI</strong></td>
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<tr>
<td>Study 1</td>
<td></td>
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<tr>
<td>BB</td>
<td>36 (15.2)</td>
<td>87 (19.9)</td>
<td>1.0</td>
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</tr>
<tr>
<td>Bb</td>
<td>109 (46.0)</td>
<td>192 (43.8)</td>
<td>1.39</td>
<td>0.87–2.23</td>
<td>0.17</td>
</tr>
<tr>
<td>bb</td>
<td>92 (38.8)</td>
<td>159 (36.3)</td>
<td>1.42</td>
<td>0.88–2.30</td>
<td>0.16</td>
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<tr>
<td>Total</td>
<td>237 (100)</td>
<td>438 (100)</td>
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<tr>
<td>Study 2</td>
<td></td>
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</tr>
<tr>
<td>BB</td>
<td>80 (13.0)</td>
<td>140 (14.7)</td>
<td>1.0</td>
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<tr>
<td>Bb</td>
<td>300 (48.6)</td>
<td>461 (48.2)</td>
<td>1.17</td>
<td>0.84–1.63</td>
<td>0.35</td>
</tr>
<tr>
<td>bb</td>
<td>237 (38.4)</td>
<td>355 (37.1)</td>
<td>1.15</td>
<td>0.82–1.62</td>
<td>0.41</td>
</tr>
<tr>
<td>Total</td>
<td>617 (100)</td>
<td>956 (100)</td>
<td></td>
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<tr>
<td>Combined</td>
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<tr>
<td>BB</td>
<td>116 (13.6)</td>
<td>227 (16.3)</td>
<td>1.0</td>
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</tr>
<tr>
<td>Bb</td>
<td>409 (47.9)</td>
<td>653 (46.8)</td>
<td>1.21</td>
<td>0.93–1.58</td>
<td>0.16</td>
</tr>
<tr>
<td>bb</td>
<td>329 (38.5)</td>
<td>514 (36.9)</td>
<td>1.23</td>
<td>0.94–1.62</td>
<td>0.13</td>
</tr>
<tr>
<td>Total</td>
<td>854 (100)</td>
<td>1394 (100)</td>
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</table>

$^a$ORs are adjusted for age, age at menarche, family history of breast cancer in first-degree relatives, region (except for the combined study), body mass index, parity/age at first birth, tobacco use, menopausal status and study (only for the combined study).

$^b$P values for comparisons of at-risk genotypes with reference homozygote genotypes.
the incidence rate of breast cancer in Ff and ff carriers would be attributable to their respective FokI genotypes.

The FokI polymorphism is located at exon 2 start codon of the VDR gene. Individuals designated F have VDR proteins missing three amino acids (424 amino acids) and those designated f have longer VDR proteins (427 amino acids) that have been shown to be functionally less efficient (reviewed in Uitterlinden et al. (2004)). Since f allele results in a less efficient VDR, the increase in breast cancer incidence associated with f allele could possibly be overturned, or at least reduced, by raising vitamin D levels, for instance through vitamin D supplementation. Interaction between VDR FokI SNP and vitamin D has been recently observed for prostate cancer (Li et al. 2007). Carriers of ff genotype with less than median circulating vitamin D were at higher risk of total and aggressive cancers while exposure to higher than median vitamin D was able to completely overcome those risks.

Since f allele results in a less efficient VDR, the increase in breast cancer incidence associated with f allele could possibly be overturned, or at least reduced, by raising vitamin D levels, for instance through vitamin D supplementation. Interaction between VDR FokI SNP and vitamin D has been recently observed for prostate cancer (Li et al. 2007). Carriers of ff genotype with less than median circulating vitamin D were at higher risk of total and aggressive cancers while exposure to higher than median vitamin D was able to completely overcome those risks.

The increase in relative risk of breast cancer with an increasing number of f alleles was significant among women without family history of breast cancer in first-degree relatives. Actually, among women declaring a family history of breast cancer in first-degree relatives, relative risk of breast cancer decreased slightly with the number of f alleles. This effect modification by family history on the relationship of f allele to breast cancer risk was observed in our two case–control studies and, in both of these, interaction was statistically significant. Such a modifying effect, if confirmed, might help to explain the apparent contradictions among results found in the literature since the strength of the relationship of the f allele to breast cancer risk seen in a given study would tend to vary according to the proportion of the study group that has a family history of breast cancer.

### BsmI and breast cancer

BsmI polymorphism is located in intron 8 of the VDR gene and is unlikely to affect VDR function. However, this polymorphism is in strong LD with the poly(A) microsatellite located in the 3’ untranslated region (UTR) that might influence either VDR mRNA stability or VDR translational activity (reviewed in Uitterlinden et al. (2004)). The variable-length poly(A) sequence has been associated with breast cancer and also with bone mineral density and height.

Eleven studies assessed BsmI association with breast cancer risk (Ruggiero et al. 1998, Ingles et al. 2000, Bretherton-Watt et al. 2001, Hou et al. 2002, Buyru et al. 2003, Guy et al. 2003, 2004, Heffler et al. 2004, Chen et al. 2005, Lowe et al. 2005, McCullough et al. 2007). Only two studies reported a significant increased risk for the BB carriers and they were respectively from a Hispanic (Ingles et al. 2000) and a Taiwanese population (Hou et al. 2002) with allele frequencies considerably different from those seen in Caucasian populations. Studies among Caucasian women are showing mixed results. Some earlier and

### Table 3

<table>
<thead>
<tr>
<th>FokI</th>
<th>With family history</th>
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<th></th>
<th>Without family history</th>
<th></th>
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<tbody>
<tr>
<td>Cases/controls</td>
<td>OR</td>
<td>95% CI</td>
<td>P</td>
<td>Cases/controls</td>
<td>OR</td>
<td>95% CI</td>
<td>P</td>
</tr>
<tr>
<td>FF</td>
<td>25/18</td>
<td>1.0</td>
<td></td>
<td>67/146</td>
<td>1.0</td>
<td></td>
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</tr>
<tr>
<td>Ff</td>
<td>71/20</td>
<td>0.56</td>
<td>0.24–1.32</td>
<td>0.18</td>
<td>84/170</td>
<td>1.01</td>
<td>0.67–1.51</td>
</tr>
<tr>
<td>ff</td>
<td>10/15</td>
<td>0.41</td>
<td>0.15–1.16</td>
<td>0.14</td>
<td>36/48</td>
<td>1.51</td>
<td>0.89–2.58</td>
</tr>
<tr>
<td>Total</td>
<td>56/59</td>
<td></td>
<td></td>
<td>187/364</td>
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</tr>
<tr>
<td>Study 2</td>
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<tr>
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<td></td>
<td>151/306</td>
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<tr>
<td>Ff</td>
<td>54/62</td>
<td>0.79</td>
<td>0.44–1.40</td>
<td>0.41</td>
<td>252/389</td>
<td>1.44</td>
<td>1.10–1.88</td>
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<tr>
<td>ff</td>
<td>19/24</td>
<td>0.78</td>
<td>0.37–1.69</td>
<td>0.54</td>
<td>93/130</td>
<td>1.65</td>
<td>1.16–2.35</td>
</tr>
<tr>
<td>Total</td>
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<td></td>
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<td>496/825</td>
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<tr>
<td>FF</td>
<td>72/65</td>
<td>1.0</td>
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<tr>
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<td>0.73</td>
<td>0.46–1.17</td>
<td>0.19</td>
<td>336/559</td>
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<td>1.02–1.58</td>
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<tr>
<td>ff</td>
<td>29/39</td>
<td>0.66</td>
<td>0.36–1.19</td>
<td>0.17</td>
<td>129/178</td>
<td>1.57</td>
<td>1.18–2.10</td>
</tr>
<tr>
<td>Total</td>
<td>176/192</td>
<td></td>
<td></td>
<td>683/1189</td>
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</table>

*ORs are adjusted for age, age at menarche, region (except in the combined study), body mass index, parity/age at first birth, tobacco use, menopausal status and study (only in the combined study).*  
*b P values for comparisons of at-risk genotypes with reference homozygote genotypes.*

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smaller studies (Bretherton-Watt et al. 2001, Guy et al. 2003, 2004, Lowe et al. 2005) reported significant increased risk for bb carriers while larger and more recent ones (Helfer et al. 2004, Chen et al. 2005) did not confirm previous associations. Although our BsmI results from both studies are non-significant, they are nevertheless showing the same pattern, perhaps suggesting a lack of statistical power. Our combined analysis suggest that if an actual increased risk exists for BsmI Bb or bb carriers, this increase would likely be small (OR = 1.22, 95% CI = 0.95–1.57) although, according to the upper limit of the confidence interval, a moderately increased risk (i.e. RR of about 1.5) cannot be excluded. Recently, the largest genome-wide association study on sporadic postmenopausal breast cancer has showed a significant association (P = 0.013) of the same order of magnitude with BsmI (rs1544410; see http://cgems.cancer.gov/data/ (Hunter et al. 2007)).

Limitations
This study faces the usual limitations of association studies between genetic markers and diseases. Since we analysed two polymorphisms chosen on the basis of previous findings and found the same association patterns in two independent studies, we believe that type I errors are less likely to explain our findings. Population stratification is the major source of confounding in this type of study (Khoury et al. 1993, Rothman & Greenland 1998) although this problem might not be as important as anticipated in North American Caucasian populations (Rothman et al. 2001, Wacholder et al. 2002, Cardon & Palmer 2003). In our two studies, the majority of women resided in the Quebec City area, where 95% of its 800 000 residents are Caucasians of French origin. We do not expect strong differences in allele frequencies within this area. Thus, it seems unlikely that our results could be due to population stratification. Non-differential misclassification bias from laboratory measurements is possible for genetic markers as for other analytical variables. Although in the absence of Hardy–Weinberg disequilibrium this is unlikely, a special case of non-differential misclassification between BsmI marker allele and an unmeasured putative susceptibility allele is also possible. This type of misclassification results from the fact that BsmI marker, being intronic, needs to be in LD with a yet unknown functional allele in order to explain a possible association between BsmI polymorphism and breast cancer. As LD between the BsmI marker and an unknown functional allele is not likely to be complete, some non-differential misclassification can occur (Khoury et al. 1993). A haplotype analysis around the 3'UTR region would help reduce this problem. The FokI polymorphism, being functional and not in LD with any other VDR polymorphism (reviewed in Uitterlinden et al. 2004), is less likely to be affected by this type of misclassification. Of note, these two types of misclassification bias would tend to underestimate true relative risks.

Conclusion
In our study population of French Canadian origin, an increasing number of FokI f allele, which was previously shown to result in a decreased efficiency of VDR, was associated with a significant increase in relative risk in a dose–response manner compatible with a co-dominant (additive) effect. This association was seen only in women without a family history of breast cancer in first-degree relatives. The study of BsmI polymorphism, however, suggested a more subtle b allele dominant risk effect, regardless of family history.

Overall, these results on VDR polymorphisms support the growing evidence for a protective effect of vitamin D on the risk of breast cancer. Future studies on the relationship of VDR FokI polymorphism and breast cancer should take into account the potential modifying effect of family history of breast cancer in first-degree relatives.

Declaration of interest
No conflict of interest that could be perceived as prejudicing the impartiality of the research reported in this article has been reported by any authors.

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