Vitamin D receptor polymorphisms (*Fok*I, *Bsm*I) 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 (*Fok*I and *Bsm*I) 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. *Fok*I and *Bsm*I genotypes were assessed. Relative risks of breast cancer were estimated by multivariate logistic regression. Compared with homozygotes for the common *F* allele (FF genotype), *Fok*I 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 *Fok*I 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). *Bsm*I 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 *Fok*I 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

Low-vitamin D intakes or circulating levels have been associated with increased breast cancer risk in several ecological (Garland *et al*. 1990, 1999, Grant 2002, 2003), case–control and cohort studies (Van't Veer *et al*. 1991, Zaridze *et al*. 1991, Knekt *et al*. 1996, Negri *et al*. 1996, John *et al*. 1999, Shin *et al*. 2002, Abbas *et al*. 2008) although not all (Graham *et al*. 1991, Simard *et al*. 1991, Levi *et al*. 2001). An inverse relationship between vitamin D/calcium intakes and breast density has also been observed (Holmes *et al*. 2001, Berube *et al*. 2004, 2005, Colangelo *et al*. 2006), as well as synchronized seasonal variations of

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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 2004*b*). 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 2004*a*).

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 (*Fok*I; rs10735810 and *Bsm*I; 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 *Fok*I and *Bsm*I 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 *Fok*I and *Bsm*I 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 *Bsm*I and *Fok*I polymorphisms after PCR amplification and digestion. Primer sequences are available upon request and PCR conditions for *Bsm*I 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 *Fok*I polymorphism, PCR amplification and digestion were done as published (Gross *et al*. 1996) except that overnight digestion with 1U of *Fok*I was used to minimize partial digests. The absence of the *Fok*I 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 *Fok*I and *Bsm*I 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 *Fok*I and *Bsm*I 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 available upon request. For each PCR, 5 μl was used regardless of the DNA concentration. Raw genotyping results were analysed using software developed in-house using VC^{++} programming language (Microsoft) that determines genotypes directly from raw fluorescence measurements for each (normal and mutated allele) allele-specific reaction. Ambiguous genotypes were reanalysed by allele-specific PCR until unambiguous results were achieved. Genotypes were automatically linked to individual DNA samples using the DNA plate bar code and the two PCR plate bar codes (one per allele). Until now, with the use of this method, duplicate analysis of 5000 genotypes from 15 different SNP assays showed an average reproducibility of 99.7% and an average rate of data rejection $<1\%$. This results in 1585 and 1584 genotyped participants for *Fok*I and *Bsm*I respectively.

The two studies, where all participants signed an informed consent form, were approved by the Research Ethics Review Board of the different institutions involved.

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 *Fok*I and *Bsm*I 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-*Fok*I: study 1, $f=0.38$; study 2, $f=0.39$ and *Bsm*I: 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).

*Fok*I 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.

*Bsm*I 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).

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 *Fok*I *f* allele was seen only in women reporting no family history (Table 3). Interaction between family history of breast cancer and *Fok*I 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 *Fok*I 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 ($P_{\text{trend}}=0.13$)

Table 1 Characteristics of women by study

	Study 1 ($n=718$)	**Study 2** ($n=1596$)
Age (years; mean ± S.D.)	52.3 ± 9.7	55.8 ± 10.5
Age (years) at menarche[a] (mean ± S.D.)	12.7 ± 1.7	12.8 ± 1.6
Family history of breast cancer[b] (%)	16.0	16.2
Body mass index (kg/m^2; mean ± S.D.)	25.0 ± 4.5	25.6 ± 4.8
Age (years) at first birth[c] (mean ± S.D.)	24.8 ± 4.3	25.6 ± 4.6
Parity[d] (%)		
Nulliparous	18.5	20.2
1–2 birth	43.9	48.3
3+birth	37.6	31.5
Smoking status[e] (%)		
Non-smoker	49.7	56.4
Former smoker	29.2	27.7
Current smoker	21.1	15.9
Postmenopausal (%)	66.8	61.1

[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 ($n=1590$).

Table 2 Restriction enzyme detecting SNP of VDR (*Fok*I and *Bsm*I) vitamin D receptor (VDR) polymorphisms and breast cancer risk

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

[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.

reached statistical significance. No effect modification by family history of breast cancer was apparent for the relationship of *Bsm*I to breast cancer (*P* interaction = 0.93; data not shown).

Discussion

Our results suggest that, within this almost exclusively Caucasian population of French origin, *Fok*I VDR polymorphism is associated with an increased risk of invasive breast cancer. The association of *Fok*I is stronger in women without a family history of breast cancer in first-degree relatives. *Bsm*I 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 *Fok*I with breast cancer risk (Curran *et al*. 1999, Ingles *et al*. 2000, Bretherton-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 (Bretherton-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 3 Restriction enzyme detecting SNP of VDR (*Fok*I) vitamin D receptor (VDR) polymorphism and breast cancer risk by family history of breast cancer

	With family history				Without family history				
***Fok*I**	**Cases/ controls**	**OR[a]**	**95% CI**	***P*[b]**	**Cases/ controls**	**OR[a]**	**95% CI**	***P*[b]**	***P* for interaction**
Study 1									
FF	25/18	1.0			67/146	1.0			
Ff	21/26	0.56	0.24–1.32	0.18	84/170	1.01	0.67–1.51	0.98	
ff	10/15	0.41	0.15–1.16	0.14	36/48	1.51	0.89–2.58	0.13	0.031
Total	56/59				187/364				
Study 2									
FF	47/47	1.0			151/306	1.0			
Ff	54/62	0.79	0.44–1.40	0.41	252/389	1.44	1.10–1.88	0.0073	
ff	19/24	0.78	0.37–1.69	0.54	93/130	1.65	1.16–2.35	0.0053	0.050
Total	120/133				496/825				
Combined									
FF	72/65	1.0			218/452	1.0			
Ff	75/88	0.73	0.46–1.17	0.19	336/559	1.27	1.02–1.58	0.030	
ff	29/39	0.66	0.36–1.19	0.17	129/178	1.57	1.18–2.10	0.0020	0.0059
Total	176/192				683/1189				

[a]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.

the incidence rate of breast cancer in Ff and ff carriers would be attributable to their respective *Fok*I genotypes.

The *Fok*I 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 *Fok*I 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.

*Bsm*I and breast cancer

*Bsm*I 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 *Bsm*I 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, Hefler *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

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 (Hefler *et al*. 2004, Chen *et al*. 2005) did not confirm previous associations. Although our *Bsm*I 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 *Bsm*I 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 *Bsm*I (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 1 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 *Bsm*I marker allele and an unmeasured putative susceptibility allele is also possible. This type of misclassification results from the fact that *Bsm*I marker, being intronic, needs to be in LD with a yet unknown functional allele in order to explain a possible association between *Bsm*I polymorphism and breast cancer. As LD between the *Bsm*I 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 *Fok*I 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 *Fok*I *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 *Bsm*I 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 *Fok*I 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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