Short Communication

The *Gc2* Allele of the Vitamin D Binding Protein Is Associated with a Decreased Postmenopausal Breast Cancer Risk, Independent of the Vitamin D Status

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Abstract

Vitamin D pathway gene polymorphisms may influence breast cancer risk by altering potential anticarcinogenic effects of vitamin D. The association between polymorphisms in the vitamin D binding protein (Gc) and postmenopausal breast cancer risk, with additional focus on the influence of serum 25-hydroxyvitamin D [25(OH)D], the biomarker for vitamin D status in humans, has not been examined thus far. We assessed the combined effects of two known functional polymorphisms in the *Gc* gene (rs4588 and rs7041), composing the phenotypic alleles *Gc1s*, *Gc1f* (combined: *Gc1*), and *Gc2*, on postmenopausal breast cancer risk and potential effect modification by 25(OH)D status in a population-based case-control study including 1,402 cases and 2,608 matched controls. Odds ratios (OR) for breast cancer risk adjusted for potential confounders were calculated for Gc genotypes. ANOVA was used to compare geometric means of serum 25(OH)D across Gc genotypes. Serum 25(OH)D concentrations in the control group significantly differed by Gc genotype, being lowest in *Gc2* allele carriers. The geometric means of 25(OH)D were 53.0, 47.8, and 40.4 nmol/L for Gc1-1, Gc2-1, and Gc2-2 genotypes, respectively (P_{trend} < 0.0001). Gc2-2 genotype was associated with a significantly decreased risk of postmenopausal breast cancer with an odds ratio (95% confidence interval) of 0.72 (0.54-0.96), compared with homozygote *Gc1s* allele carriers. No interaction between 25(OH)D status and Gc genotype was observed, nor did the association change considerably after adjustment for 25(OH)D status. Our results provide evidence for a serum 25(OH)D-independent effect of *Gc2* allele carrier status in postmenopausal breast cancer. (Cancer Epidemiol Biomarkers Prev 2008;17(6):1339–43)

Introduction

Antiproliferative effects of vitamin D in various cell types including normal and malignant breast cells, by influencing cell differentiation, cell growth, and apoptosis, are well established (1-3). In addition, both vitamin D status, as measured by serum 25-hydroxyvitamin D [25(OH)D] or 1,25-dihydroxyvitamin D [1,25$(OH)_2$D], the biologically active form of vitamin D, and polymorphisms in vitamin D pathway genes have been associated with breast cancer risk (4-6). A key protein in vitamin D metabolism is the vitamin D binding protein, known as ''group-specific component'' (Gc). Gc is the major transport protein of vitamin D metabolites to different target organs. Additionally, deglycosylation converts Gc to a potent macrophage-activating factor (GcMAF) that stimulates phagocytotic activity of macrophages during inflammation (7). More recently, antitumor activities of GcMAF in mice (8) as well as immunotherapeutic properties in metastatic breast cancer patients have been described (9).

There are three common phenotypic alleles in the Gc protein (*Gc1s*, *Gc1f*, and *Gc2*) differing by combinations of two nonsynonymous single-nucleotide polymorphisms, rs4588 and rs7041, and by their glycosylation pattern in the Gc protein (galactose and sialic acid in both Gc1s and Gc1f; galactose only in Gc2; ref. 10; Supplementary Table S1). These phenotypic alleles have been associated with differences in Gc and serum 25(OH)D concentration as well as affinity of Gc to vitamin D metabolites. Compared with *Gc2* alleles, *Gc1s* and *Gc1f* were associated with higher levels of Gc and serum 25(OH)D concentration as well as higher affinity of Gc to vitamin D metabolites (11-13). Additionally, Gc phenotype has been associated with premenopausal fracture risk, emphasizing its effect on osteoporosis (14). However, to our knowledge, only one study thus far assessed the association of *Gc* alleles with breast cancer risk, reporting no association when considering the two single-nucleotide polymorphisms rs7041 and rs4588

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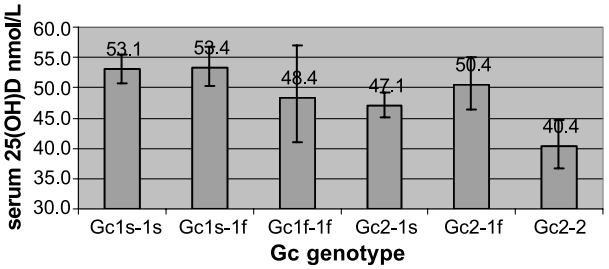

Figure 1. Mean serum 25(OH)D concentrations in the control group by Gc genotypes. *Columns,* geometric means; *bars,* 95% CI. $P < 0.05$, pairwise comparison between the genotypes 1s-1s and 2-1s, 1s-1s and 2-2, 1s-1f and 2-1s, 1s-1f and 2-2, 2-1s and 2-2, and 2-1f and 2-2 (ANOVA, Scheffé test).

separately (15). No study has thus far assessed these two polymorphisms together and additionally taken the vitamin D status into account. We recently reported an inverse association between serum 25(OH)D and postmenopausal breast cancer risk in a German case-control study (4). We now investigated the association between *Gc* alleles and postmenopausal breast cancer risk in the same study population with additional focus on potential interaction by serum 25(OH)D and influence of the Gc genotypes on 25(OH)D status.

Materials and Methods

We used a large population-based case-control study (MARIE study) carried out in the city of Hamburg and the Rhein-Neckar-Karlsruhe region, Germany. The study was approved by the ethics committees of the University of Heidelberg and the University of Hamburg. Cases were eligible if they had a histologically confirmed primary invasive or *in situ* breast cancer diagnosed between 01/01/2001 and 30/09/2005 in Hamburg and between 01/08/2002 and 31/07/2005 in the Rhein-Neckar-Karlsruhe region, were of ages 50 to 74 years, and a resident of one of the study regions. Of the 5,970 eligible patients who could be contacted, 3,919 (65.6%) participated. Two controls per case were randomly selected from population registries matched by year of birth and study region to the cases. Of the 17,093 controls who met the inclusion criteria, 7,421 (43.4%) participated. Further study details were published previously (4).

Information on sociodemographic factors and potential breast cancer risk factors was obtained by personal interview. Menopausal status was assigned as described previously (4). In total, 3,464 invasive or *in situ* breast cancer cases and 6,657 controls were classified as postmenopausal, including 1,559 cases and 3,008 controls from the Rhein-Neckar-Karlsruhe region.

Postmenopausal participants of the Rhein-Neckar-Karlsruhe region with DNA samples were included in this analysis, composed of 1,402 (89.9%) cases and 2,608 (86.7%) controls.

We measured serum 25(OH)D in a subset of 1,391 postmenopausal cases and 1,365 randomly selected postmenopausal controls matched on year of birth and time of blood collection using the OCTEIA 25-hydroxyvitamin D enzyme immunoassay (IDS) as previously described in more detail (4).

Genotyping was done in collaboration with the molecular laboratory BioGlobe GmbH using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry based on Sequenom's hME and iPlex technology.

Women were allocated to three common *Gc* alleles (haplotypes), *Gc1s, Gc1f, Gc2*, by their respective single-nucleotide polymorphism in rs7041 (Asp416Glu, T/G) and rs4588 (Thr420Lys, C/A) as follows: *Gc1f*, T and C; *Gc1s*, G and C; and *Gc2*, T and A, at single-nucleotide polymorphism rs7041 and rs4588, respectively (Supplementary Table S1). The corresponding combined genotypes were Gc1s-1s, Gc1s-1f, Gc1f-1f, Gc2-1s, Gc2-1f, and Gc2-2 (Supplementary Table S2). When combining *Gc1s* and *Gc1f* allele carriers, the combined genotypes were Gc1-1, Gc2-1, and Gc2-2.

We assessed the association of Gc combined genotype with postmenopausal breast cancer risk, taking the most frequent genotype as the reference category, by means of logistic regression with stratification by year of birth and additional stratification by time of blood collection (quarters of the year) in models assessing interaction with 25(OH)D. Odds ratios (OR) and corresponding 95% confidence intervals (95% CI) are presented. The following variables were included in the multivariate model: age at menopause (<47, 47-51, 52-55, ≥56 y, unknown), body mass index (<22.5, 22.5-<25, 25-<30, ≥30 kg/m^2), education level (low, middle, high), first-degree family history of breast cancer (yes, no, unknown), history of benign breast disease (yes, no), number of pregnancies (≥28th week; 0, 1, 2, ≥3), age at menarche (<12, 12-14, ≥15), breast-feeding history (ever, never), total number of mammograms (0, 1-4, 5-9, ≥10, unknown), smoking status (never, past, current), and use of menopausal hormone therapy (never, past, current).

Statistical interaction was evaluated with the likelihood ratio test by including an interaction term of the dichotomous genotype variable (*Gc2* carriers versus noncarriers) and potential interaction variables [continuous variable for 25(OH)D] in the model.

In an ANOVA, mean values of the log-transformed 25(OH)D variable were compared across genotypes. Between-group comparisons were adjusted for multiple comparisons using Scheffé test.

All tests were two-sided with a significance level of $P \leq 0.05$. Calculations were conducted with SAS 9.1 (SAS Institute).

Results

Descriptive characteristics of the study population have previously been described (4). Median 25(OH)D concentration was 44.9 nmol/L for cases and 51.5 nmol/L for controls. No deviation from Hardy-Weinberg equilibrium was observed for rs4588 and rs7041 ($P = 0.60$ and $P = 0.56$ in the controls, respectively). The observed allele frequencies in the controls were comparable to those reported in the dbSNP database for Caucasians. Serum 25(OH)D concentrations differed significantly by genotype in the control group, being lowest in homozygote *Gc2* carriers (Fig. 1). When combining *Gc1s* and *Gc1f* allele carriers, geometric mean 25(OH)D concentrations

Table 1. ORs for postmenopausal breast cancer by Gc genotype in the vitamin D binding protein

Gc genotype	Cases	Controls	Crude model*	Adjusted model 1 †	Adjusted model 2 ‡
	n (%)	*n* (%)	OR (95% CI)	OR (95% CI)	OR (95% CI)
	1,402	2,608			
Gc1s-1s	472 (33.7)	837 (32.1)	1	1	1
Gc1s-1f	255 (18.2)	450 (17.2)	1.00 (0.83-1.21)	1.00 (0.82-1.22)	1.05 (0.83-1.32)
Gc1f-1f	39 (2.8)	65 (2.5)	1.07 (0.71-1.61)	1.00 (0.65-1.52)	1.03 (0.62-1.70)
Gc2-1s	433 (30.9)	844 (32.4)	0.92 (0.78-1.08)	0.90 (0.76-1.07)	0.86 (0.71-1.05)
Gc2-1f	117 (8.3)	216 (8.3)	0.96 (0.75-1.24)	0.96 (0.74-1.25)	0.85 (0.63-1.15)
Gc2-2	86 (6.1)	196 (7.5)	0.79 (0.60-1.04)	0.72§ (0.54-0.96)	0.76 (0.54-1.07)
				P_{trend} = 0.04	
Gc1-1	766 (54.7)	1,352 (51.8)	1	1	1
Gc2-1	550 (39.2)	1,060 (40.7)	0.93 (0.81-1.06)	0.91 (0.79-1.05)	0.85 (0.72-1.00)
Gc2-2	86 (6.1)	196 (7.5)	0.79 (0.60-1.03)	0.72§ (0.54-0.95)	0.75 (0.54-1.04)
Gc2-1/2-2			0.91 (0.79-1.03)	0.88 (0.77-1.01)	0.84§ (0.72-0.98)
				P_{trend} = 0.02	

* Conditional logistic regression stratified by year of birth.
† Conditional logistic regression stratified by year of birth adjusted for age at menopause, first-degree family history of breast cancer, history of benign breast disease, number of pregnancies (≥28th wk), age at menarche, breast-feeding history, total number of mammograms, use of hormone therapy, body mass index, education level, and smoking status.
‡ Adjustment as in model 1 with additional stratification by time of blood collection and additional adjustment on 25(OH)D status as continuous variable; 25(OH)D status was available only for 1,391 cases and 1,365 controls.
§ $P < 0.05$.

were 53.0, 47.8, and 40.4 nmol/L for Gc1-1, Gc2-1, and Gc2-2 genotypes, respectively, with a significant linear trend by increasing number of *Gc2* alleles ($P_{trend} < 0.0001$; data not shown). Adjustment on time of blood collection and body mass index did not change the mean 25(OH)D estimates.

We observed a significant inverse association between the Gc genotype and postmenopausal breast cancer risk comparing homozygote carriers of the *Gc2* allele with the most frequent genotype, Gc1s-1s [OR, 0.72; 95% CI, 0.54-0.96; P_{trend} = 0.04] (Table 1). When compared with noncarriers, *Gc2* carriers had an OR of 0.88 (95% CI, 0.77-1.01). Adjustment for serum 25(OH)D status did not change the risk estimates substantially (Table 1). We did not observe a significant interaction between serum 25(OH)D and Gc genotype ($P_{interaction}$ = 0.22).

Because vitamin D possibly exerts its anticarcinogenic activities via the estrogen pathway, we assessed possible differential effects by receptor status of the tumor. We did not observe a significant interaction between Gc genotype and estrogen receptor (ER) status of the tumor ($P_{interaction}$ = 0.56 in a case-only model). However, a significant inverse association for postmenopausal breast cancer comparing *Gc2* carriers with noncarriers was observed only for progesterone receptor (PR)–positive tumors [OR, 0.81 (95% CI, 0.69-0.96) and 1.03 (95% CI, 0.84-1.27), for PR^+ and PR^- tumors, respectively; $P_{interaction}$ = 0.006; Table 2]. The effect modification was independent of the ER status, with an inverse association in both ER^+/PR^+ and ER^-/PR^+, and no association in ER^+/PR^- and ER^-/PR^- tumors (data not shown).

Discussion

In this population-based case-control study, we observed a significantly reduced risk of postmenopausal breast

Table 2. ORs for postmenopausal breast cancer by Gc genotypes in the vitamin D binding protein gene according to ER and PR status of the tumor

Gc genotype	Gc1-1	Gc2-1	Gc2-2	Gc2-1/2-2
ER^+ tumors				
n (Ca/Co)	540/1,352	387/1,060	62/196	
OR (95% CI)	1	0.92 (0.78-1.08)	0.72 (0.52-0.98)	0.88 (0.76-1.03)
ER^- tumors				
n (Ca/Co)	168/1,352	120/1,060	19/196	
OR (95% CI)	1	0.91 (0.71-1.18)	0.75 (0.45-1.26)	0.89 (0.69-1.13)
PR^+ tumors				
n (Ca/Co)	477/1,352	324/1,060	44/196	
OR (95% CI)	1	0.86 (0.73-1.02)	0.57 (0.40-0.81)	0.81 (0.69-0.96)
PR^- tumors				
n (Ca/Co)	229/1,352	184/1,060	37/196	
OR (95% CI)	1	1.03 (0.83-1.27)	1.07 (0.73-1.59)	1.03 (0.84-1.27)

NOTE: Conditional logistic regression stratified by year of birth adjusted for age at menopause, first-degree family history of breast cancer, history of benign breast disease, number of pregnancies (≥28th wk), age at menarche, breast-feeding history, total number of mammograms, use of hormone therapy, body mass index, education level, and smoking status.
Data on ER and PR status were available for 1,296 and 1,295 cases, respectively. $P_{interaction}$ = 0.56 and 0.006 for ER and PR status in a case-only model, respectively.
Abbreviations: Ca, cases; Co, control.

cancer in homozygote carriers of the *Gc2* allele in the vitamin D binding protein. These results were independent of serum 25(OH)D because adjustment for 25(OH)D concentration did not affect the risk estimates and interaction between genotype and 25(OH)D was not observed. Thus, homozygote carriers of the *Gc2* allele, who have low serum 25(OH)D levels, showed a reduced risk of breast cancer despite previous observations of an inverse association between serum 25(OH)D and breast cancer risk in the same study population (4). There are indeed data to support this observation. In a cross-sectional study, women with the Gc2-2 phenotype also had lower concentrations of 25(OH)D as compared with the Gc1-2 or Gc1-1 phenotype (13). However, these women showed no evidence of vitamin D insufficiency (e.g., increased plasma parathormone level) and had a lower risk of bone fracture (14). Based on these results, the authors proposed to use a lower 25(OH)D plasma level for defining vitamin D sufficiency in women with Gc2-2 as compared with the Gc1-2 or Gc1-1 phenotype.

The importance of the vitamin D binding protein in breast carcinogenesis was recently described. Rowling et al. (16) reported an endocytotic uptake of the vitamin D binding protein-25(OH)D-complex in breast cancer cells that was correlated to the activation of the vitamin D receptor pathway, which in turn leads to anticarcinogenic action of vitamin D (17). It is possible that women carrying *Gc2* allele(s) have a higher uptake of Gc-25(OH)D complex or a better transport to the target organs (e.g., breast tissue) and, therefore, a reduced breast cancer risk.

An anticarcinogenic mechanism different from the activation of the vitamin D receptor pathway lies in the potential of Gc to convert to GcMAF, a potent activator of macrophages (7). GcMAF has been shown to be effective as an antitumorigenic drug in metastatic breast cancer patients (9) and to inhibit tumor growth and increase apoptotic activity in pancreatic cell lines (8). The conversion of Gc to GcMAF requires a deglycosylation process mediated by β-galactosidase and sialidase (i.e., the removal of galactose and sialic acid residues; ref. 18). It is therefore of interest that, compared with Gc1s and Gc1f, the conversion of Gc2 protein (containing galactose only) to GcMAF requires solely β-galactosidase activity (19). We observed a decreased breast cancer risk only in *Gc2* allele carriers but no differences in risk estimates between *Gc1s* and *Gc1f* carriers with the same glycosylation pattern. Thus, we hypothesize that different glycosylation patterns in the *Gc* alleles may explain the observed 25(OH)D-independent decrease in breast cancer risk. Both qualitative and quantitative differences between Gc2MAF and Gc1MAF are possible.

One previous study assessed the two polymorphisms composing the *Gc* alleles and found no association with postmenopausal breast cancer risk (15). However, the study did not have adequate power to assess the effects of the different *Gc* alleles and did not take the vitamin D status into account.

We also looked at the differential effects of the Gc genotype on breast cancer by receptor status of the tumor. The anticarcinogenic effects of vitamin D could be mediated via the estrogen pathway by down-regulation of the ER and, thus, attenuating estrogenic bioresponses like cell growth (20, 21). No interaction was observed between the ER of the tumor and Gc genotypes. However, the observed inverse association in progesterone-positive, but not progesterone-negative, tumors deserves further investigation.

Strengths of our study are the large sample size, the adjustment for all potential breast cancer risk factors, and the consideration of serum 25(OH)D status. Limitations due to the retrospective case-control design are negligible because genotype distribution is not prone to selection or recall bias. If the probability of being carriers of the risk alleles was associated with stage of diagnosis, the lower participation among cases with later stage at diagnosis would have biased our results. However, the genotype distribution of the two analyzed polymorphisms did not differ significantly by stage of the tumor. The 25(OH)D concentrations could be affected by diagnosis or treatment. The median difference (25th-75th percentile) between time of diagnosis and time of blood collection in the cases was 80 (14-260) days. However, in a sensitivity analysis excluding patients with blood samples taken within 6 months of diagnosis, we observed negligible changes in the risk estimates of the association between 25(OH)D levels and breast cancer risk (4). A notable change in 25(OH)D concentration after chemotherapeutic treatment has also not been observed previously (22, 23).

We selected known functional variants with potential effect on vitamin D metabolism; however, our findings on breast cancer risk need verification in further studies.

In summary, we observed a significant inverse association between genotypes in the vitamin D binding protein and postmenopausal breast cancer, comparing homozygote *Gc2* with homozygote *Gc1s* allele carriers. Additionally, the *Gc2* allele was associated with a decreased serum 25(OH)D concentration. Our data suggest a protective effect for *Gc2* allele carrier status in postmenopausal breast cancer that is independent of serum 25(OH)D status. A possible anticarcinogenic mechanism of the Gc protein is provided by its potential conversion to GcMAF, a macrophage-activating factor known to display anticarcinogenic activities. However, further research on the influence of Gc genotypes on Gc protein and GcMAF activity is necessary.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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