BRCA2 Promoter Hypermethylation in Sporadic Breast Cancer

Rémy Bosviel, Julie Durif, Jiaoli Guo, Mourad Mebrek, Fabrice Kwiatkowski,
Yves-Jean Bignon, and Dominique J. Bernard-Gallon

Dear Editor:

Breast cancer is a multifactorial disease. It is the first cause of mortality per cancer for the woman in the world (Jemal *et al.*, 2011). The implication of specific genes, such as *BRCA1* and *BRCA2* tumor suppressor genes, has been shown in mammary carcinogenesis. In sporadic breast cancers, specific modifications of *BRCA1* and *BRCA2* mRNA expression have been reported too (Bernard-Gallon *et al.*, 1999; Bieche *et al.*, 1999). Epigenetic modifications such as promoter hypermethylation of the oncosuppressor genes *BRCA1* and *BRCA2* can play a role in the oncogenesis of cancer (Esteller *et al.*, 2000). Indeed, hypermethylation of the CpG islands in the promoters of these genes involve their inactivation and therefore a higher risk of developing a tumor (Baylin and Ohm, 2006; Moelans *et al.*, 2011; Rahmatpanah *et al.*, 2009).

We have compared the DNA methylation rates of the *BRCA1* and *BRCA2* gene promoters using DNA isolated from blood samples from COSA (Cancer d'Ovaire et du Sein en Auvergne) patients suffering from breast or ovarian cancer and from a population of healthy women. To this aim, the QAMA method (Quantitative Analysis of Methylated Alleles) was used (Zeschnigk *et al.*, 2004) and adapted to *BRCA1* and *BRCA2* genes (Bosviel *et al.*, 2011). This method is based on bisulfite conversion of the nonmethylated cytosines and an analysis by qPCR using Taqman minor groove binder probes specific for methylated or nonmethylated target sites after conversion. Percentage of methylation is then obtained by calculating the difference between C_T values of the methylated DNA targeting probe and the nonmethylated DNA targeting probe (ΔC_T) and reporting the obtained values on a standard curve.

In a previous work, we reported that *BRCA1* methylation is significantly decreased in ovarian cancer by comparison with the control group. The comparison between the two different populations did not show any significant difference regarding *BRCA2* methylation but exhibited a trend in the decrease of *BRCA2* promoter methylation in peripheral blood DNA of sporadic ovarian cancer (Bosviel *et al.*, 2011).

Then we demonstrated a trend toward *BRCA1* promoter hypermethylation in PBCs of sporadic breast cancer patients by comparison with controls (Bosviel *et al.*, 2012). *BRCA1* promoter methylation in PBCs corresponded to 47.1% with CI 95% [46.1; 48.1] in breast cancer patients and to 45.9% with CI

95% [45.0; 46.8] in controls. Association between methylation level and clinicopathological features were evaluated using statistical tests. *BRCA1* promoter methylation in PBCs increased significantly in breast cancer patients by comparison with controls, with the age over 70 years old ($p=0.022$), in post menopausal status ($p=0.013$), with a BMI < 20 ($p=0.0095$), or with a WHR ≤ 76.8 ($p=0.0027$). We also found an association of increased *BRCA1* promoter methylation in PBCs with ACA/ACA genotype for the SNP Thr⁵⁹⁴Thr in *ESR* (estrogen receptor), known to be associated with breast cancer risk ($p=0.092$), due to the reduced presence of this genotype in this breast cancer case-control study.

Within this study, the objective was to compare the methylation of the CpG islands present in the *BRCA2* promoter in the same population of women suffering from breast cancer compared to the control population. In total, 873 breast samples belonging to COSA and 980 control samples were converted and the methylation rates measured. The complete database of this study is available as supplementary data (supplementary data are available online at www.liebertonline.com/omi).

BRCA2 promoter methylation mean in PBCs is 16.9% (CI95% [16.3; 17.4]) in breast cancer patients and 16.2% (CI95% [15.7; 16.8]) in controls. The statistical analysis of the mean methylation rates obtained for the *BRCA2* promoter did not reveal a significant difference ($p=0.1$) between the two populations (Fig. 1).

Significant differences in methylation rates between patients and healthy women were, however, obtained for different subclasses (Table 1). It reached 17.3% in breast cancer patients older than 70 years by comparison with control patients (14.7%) with $p=0.016$. In the subclass with an early menopause (before 48 years), the level was respectively 16.6% in breast cancer patients versus 15.4% in control patients ($p=0.028$).

Then, breast cancer patients showing a normal BMI (Body Mass Index) [20–25] exhibited a *BRCA2* promoter methylation in PBCs of 17.6% versus 16.5% for control patients ($p=0.019$).

Concerning the increase in the WHR (Waist-to-Hip Ratio) [81.7%–87.5%], the *BRCA2* promoter methylation in PBCs was 17.1% in COSA patients by comparison to control patients (15.4%) with $p=0.0046$. So, an android distribution of fat tissue revealed a higher methylation rate of the *BRCA2* promoter region.

Patients	Mean	Standard Deviation	95% Confidence Interval	p
Breast Cancer (n=873)	16.9	8.0	[16.3 ; 17.4]	0.10
Control (n=980)	16.2	8.1	[15.7 ; 16.8]	

FIG. 1. Average percentage of methylation of the promoter of *BRCA2* in DNA from control patients and COSA ($p=0.10$).

TABLE 1. DEMOGRAPHICS OF BREAST CANCER PATIENTS AND CONTROLS AND THEIR ASSOCIATION WITH *BRCA2* PROMOTER METHYLATION IN PERIPHERAL BLOOD CELLS

	Breast Cancer Patients					Control					Patients/Controls Comparison p=0.10
	Number 873	Average 16.9	SD 8.0	95%CI 16.3–17.4	Comparison in Category	Number 980	Average 16.2	SD 8.1	95%CI 15.7–16.8	Comparison in Category	
Age											
< 50 years	252	17.2	7.6	16.3–18.1	p=0.66	142	16.8	8.2	15.4–18.1	p=0.23	p=0.39
[50–60[years	241	16.6	7.8	15.6–17.6		437	16.5	8.0	15.7–17.2		p=0.57
[60–70[years	221	16.4	7.9	15.4–17.5		304	16.2	8.3	15.2–17.1		p=0.45
≥ 70 years	159	17.3	8.8	16.0–18.7		97	14.7	7.5	13.2–16.2		p=0.016
Menopausal status											
Premenopausal	265	17.1	7.8	16.2–18.0	p=0.52	246	16.3	8.0	15.3–17.3	p=0.72	p=0.18
Postmenopausal	552	16.7	8.0	16.1–17.4		734	16.2	8.1	15.6–16.8		p=0.24
Menopausal Age											
≤ 48 years	170	16.6	7.3	15.5–17.7	p=0.63	196	15.4	8.4	14.2–16.6	p=0.076	p=0.028
[48–50] years	117	16.9	7.6	15.5–18.3		186	16.7	8.6	15.5–18.0		p=0.45
[50–53] years	136	15.6	7.6	14.3–16.8		191	15.8	7.5	14.8–16.9		p=0.72
> 53 years	123	18.0	9.4	16.4–19.7		159	17.2	7.8	16.0–18.4		p=0.62
BMI											
< 20	97	16.9	8.1	15.3–18.5	p=0.091	134	15.4	8.1	14.0–16.7	p=0.29	p=0.094
[20–25[	394	17.6	8.1	16.8–18.4		541	16.5	8.0	15.8–17.2		p=0.019
[25–30[	216	15.9	7.5	14.9–16.9		220	16.4	8.4	15.3–17.5		p=0.82
≥ 30	106	16.3	7.6	14.9–17.8		85	15.5	7.8	13.9–17.2		p=0.48
WHR											
≤ 76.8	105	17.2	8.4	15.6–18.8	p=0.83	320	17.1	8.6	16.2–18.1	p=0.052	p=0.74
[76.8–81.7]	163	16.7	7.4	15.6–17.9		266	15.7	7.6	14.8–16.6		p=0.13
[81.7–87.5]	196	17.1	7.6	16.0–18.2		238	15.4	7.8	14.4–16.4		p=0.0046
> 87.5	272	16.6	8.0	15.7–17.6		144	16.9	8.4	15.6–18.3		p=0.80

BMI, Body Mass Index; WHR, Waist-to-Hip Ratio; 95%CI, 95% Confidence Interval; SD, Standard deviation.

For 2 populations with a Gaussian distribution. Student t test was used for equal variance population; otherwise, Z-score test ($n \geq 30$) was used. To compare more than 2 populations, variance analysis was performed. Finally, Kruskal-Wallis test was performed for non-Gaussian population comparisons.

TABLE 2. RELATIONSHIP BETWEEN CLINICOPATHOLOGICAL CHARACTERISTICS OF TUMORS AND *BRCA2* PROMOTER METHYLATION IN PBCs OF BREAST CANCER PATIENTS

	Breast Cancer Patients				Comparison in the Category ^a
	Number 873	Average 16.9	SD 8.0	95%CI 16.3–17.4	
SBR degrees					
1	244	17.1	8.9	15.9–18.2	$p=1.00$
2	290	16.7	8.0	15.8–17.6	
3	129	16.5	7.2	15.3–17.8	
Histological type					
IDC	599	16.7	7.9	16.1–17.4	$p=0.80$
ILC	105	17.1	7.1	15.7–18.4	
other	168	17.0	8.7	15.7–18.3	
Tumor size					
Infraclenic	127	16.9	7.5	15.6–18.2	$p=0.92$
<2 cm	157	16.2	7.7	15.0–17.4	
2–5 cm	150	16.2	6.6	15.2–17.3	
>5 cm	26	16.7	8.2	13.4–20.0	
Epidermal invasion	26	16.8	9.3	13.2–20.4	
Lymph node metastasis					
N0	306	16.5	7.1	15.7–17.3	$p=0.20$
N1–N2	111	15.8	7.9	14.3–17.3	

95%CI, 95% confidence interval; SD, standard deviation; SBR, Scarff-Bloom-Richardson; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; N0, no invasive lymph node, and N1–N2, invasive lymph nodes. ^aStudent *t* test.

FIG. 2. ROC curve analysis using DNA samples extracted from PBCs for discriminating between breast cancer patients and normal subjects based on methylation pattern of the *BRCA2* gene.

TABLE 3. ROC CURVE ANALYSIS OF DNA SAMPLES EXTRACTED FROM PBCs BASED ON METHYLATION PROPORTION OF THE *BRCA2* GENE

AUC*	0.53
Sensitivity	52.50%
Specificity	55.80%
Positive predictive value	51.40%
Negative predictive value	56.90%
Chi ²	12.68
p Chi ²	0.00037
Relative Risk (RR)	1.19
Asymptotic 95% CI**	[1.08; 1.31]
Odd Ratio (OR)	1.39
Asymptotic 95% CI	[1.16; 1.67]

*AUC, area under the curve; **CI, confidence interval (lower-upper bound).

Conversely, relationship between clinicopathological characteristics of tumors in subclasses and *BRCA2* promoter methylation in PBCs of breast cancer patients demonstrated no statistical significant difference for any of the analyzed clinical parameters (Table 2).

Sensitivity and specificity of the blood-based assay to distinguish breast cancer cases from controls, using *BRCA2* methylation, were calculated. Reliability of the methylation of a gene such as *BRCA2* to serve as a sensitive and specific blood-based breast cancer test was assessed with significant different methylation patterns between cancerous and normal PBCs. To evaluate the applicability of *BRCA2* methylation in circulating DNA from PBCs as a marker for breast cancer, receiver operating characteristic (ROC) curve analysis was used (Fig. 2). The best cut-off point (16.21% of methylation) and the corresponding sensitivity (52.3%), specificity (55.8%), area under curve (AUC = 0.530) and confidence interval were calculated (Table 3). The positive predictive value (PPV) was 51.4% and negative predictive value (NPV) was 56.9% in case of a cut-off point at 16.21% for the methylation. The Chi² test (12.68) between the risk and the disease was found significant ($p = 0.00037$). The Relative Risk (RR = 1.19; 95% CI [1.08; 1.31]) and the Odd Ratio (OR = 1.39; 95% CI [1.16; 1.67]) to develop the disease by comparison with the reference fixed at 1, were calculated and were found included in their respective asymptotic 95% CI.

The presenting data are promising for development of a blood-based screening method for breast cancer that relies on pathologic methylation changes, corroborating other results with different genes (Radpour *et al.*, 2011). Further studies comparing tissue specific and blood-based methylation markers might provide valuable information as prognostic and predictive markers for breast cancer, as well as for developing novel targeted therapeutic strategies.

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Mourad Mebrek, Fabrice Kwiatkowski,
Yves-Jean Bignon, and
Dominique J. Bernard-Gallon

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Author Disclosure Statement

The authors declare that no competing financial interests exist.

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Address correspondence to:

Dr. Yves-Jean Bignon
CBRV

Centre Jean Perrin
Département d'Oncogénétique
28 place Henri Dunant, BP 38
63001 Clermont-Ferrand
France

E-mail: Yves-Jean.Bignon@cjp.fr