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Short communication

Peripheral blood DNA methylation detected in the *BRCA1* or *BRCA2* promoter for sporadic ovarian cancer patients and controls

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ABSTRACT

Background: Ovarian cancer is located at the fifth rank of female cancers. Different risk factors including genetic factors with *BRCA1* and *BRCA2* genes played an important role in the etiology of the ovarian cancer. In most of sporadic ovarian cancer, variation in the expression of *BRCA1* and *BRCA2* genes was observed and it could be a consequence of epigenetic modifications. This work aimed to study methylation at CpG islands within the promoter of the *BRCA1 and BRCA2* genes in sporadic ovarian cancers.
Methods: For this, we conducted a case-control study consisted of 51 ovarian cancer cases with no *BRCA* mutation and 349 healthy women. All participants came from the Auvergne region in France. Genomic DNA was extracted from peripheral blood cells (PBCs) and we used the Quantitative Analysis of Methylated Alleles (QAMA) to estimate the per cent of methylation in the *BRCA1* and *BRCA2* promoters.
Results: 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.
Conclusions: These results may have implications in better understanding the underlying epigenetic mechanisms in *BRCA1* and *BRCA2* oncosuppressors in sporadic ovarian cancer.

1. Introduction

Ovarian cancer is one of the main causes of death among gynecological malignancies [1]. Methylation is an important silencing mechanism of breast and ovarian cancer susceptibility gene 1 (*BRCA1*) expression in sporadic ovarian cancer. Less is known in methylation about ovarian cancer susceptibility gene 2 (*BRCA2*).

Aberrant methylation may contribute to the disruption of key biological pathways during the progression of ovarian cancer to the drug–resistant phenotype [2–4].

Methylation-specific PCR (MSP) is a the technique frequently used for methylation analysis [5,6]. The methylation is discriminated by PCR in methylated and unmethylated target DNA. Many improvements appeared like the MethyLight [7]. Here, we used the assay presented by Zeschnigk et al., [8] which is a quantitative MethyLight based on minor groove binder (MGB) technology, called QAMA (Quantitative analysis of methylated alleles).

These results taken together led us to hypothesize that *BRCA1* and *BRCA2* promoter methylation in PBCs may indicate the propensity of a woman's normal ovarian tissue to have *BRCA1* and *BRCA2* promoter methylation and that such would become susceptible to ovarian cancer pathogenesis. This would mean that *BRCA1* and *BRCA2* promoter methylation in PBCs could constitute a risk factor for ovarian cancer. In the case-control study presented here, we, there investigated whether *BRCA1* and *BRCA2* promoter methylation in PBCs is associated with risk of cancer, especially ovarian cancer with *BRCA1* and *BRCA2* promoter methylation.

2. Patients and methods

2.1. Patients and controls

Fifty-five women aged 24–84 years who had been diagnosed with ovarian cancer with no *BRCA* mutation were enrolled in the COSA (Breast and Ovarian Cancer in Auvergne) program between November 1996 and November 1999 in different hospitals within the

Abbreviations: PBCs, peripheral blood cells; QAMA, quantitative analysis of methylated alleles; COSA, breast and ovarian cancer in Auvergne; ARDOC, organized regional screening program association; MGB, minor grove binder; FAM, fluorescein amidite; SEM, standard error to mean.

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Auvergne region of France. A control population (n = 349) was gathered in 2005 and 2006 in a mammographic screening center. The majority of volunteers were women who went for screening in response to the Organized Regional Screening Program Association (ARDOC). This program consisted of inviting all women from the Auvergne region for a free mammography read by two independent radiologists. Eligible controls were women with no previous history of cancer, no more than one first degree relative breast or ovarian cancer and resident in Auvergne. Written informed consent was obtained from all the cases and the controls. A blood sample was collected.

2.2. DNA extraction

Genomic DNA samples were extracted from control patients and from ovarian cancer patients using DNA extraction kit by Euromedex according to the manufacturer's protocol (Euromedex, Souffelweyersheim, France).

2.3. Bisulfite treatment

Bisulfite treatment of denatured DNA that converts all unmethylated cytosines to uracil, leaving methylated cytosines unaltered [9] was carried out using the methylSEQr™ Bisulfite Modification Kit (Applied Biosystems) following manufacturer's instructions.

2.4. Quantitative analysis of methylated alleles (QAMA)

Real-time PCR-based QAMA assay was described previously by Zeschnigk *et al.*[8]. PCR was performed using a 96-well optical tray with optical adhesive film at a final reaction volume of 20 µl. Samples contained 10 µl of TaqMan® Universal PCR Master Mix II, No AmpErase® UNG (uracil-N-glycosylase), 8 µl of bisulfite-treated DNA, an additional 5 U of FastStart Taq DNA Polymerase (Roche), 2.5 µM each of the primers and 150nM of the fluorescently labeled methylated and unmethylated *BRCA1* or methylated and unmethylated *BRCA2* probes. Initial denaturation at 95 °C for 10 min to activate DNA polymerase was followed by 40 cycles of denaturation at 95 °C for 15 s and annealing and extension at 60 °C for 1 min (Applied Biosystems, 7900HT, Real-Time PCR System). Primer and probe sequences were selected with the help of Primer Express software (ABI). PCR primers were designed to amplify the bisulfite-converted sense strand of the CpG Island *BRCA1* promoter sequence or antisense strand of the CpG Island *BRCA2* promoter sequence, lacking any known nucleotide polymorphisms. The software designs primers with a melting temperature (*Tm*) of 58–60 °C and probes with *Tm* value of 68°–69 °C. The *Tm* of both primers should be equal. The amplicon sizes were 79 bp for *BRCA1* and 87 bp for *BRCA2*. Primer and probe sequences were shown in Table 1. The primer binding sites lack CpG dinucleotides and, therefore, the nucleotide sequences in methylated and unmethylated DNA are identical after bisulfite treatment. Consequently, it is possible to amplify both alleles in the same reaction tube with one primer pair. Methylation discrimination occurs during probe hybridization by the use of two different MGB Taqman® probes. The binding site of the *BRCA1* and *BRCA2* MGB Taqman® probes both cover 2 CpG dinucleotides. We used a VIC-labeled MGB Taqman® probe that specifically hybridizes to the sequence derived from the methylated allele, and a FAM-labeled MGB Taqman® probe that binds to the sequence generated from the unmethylated allele (Fig. 1). The amount of FAM and VIC fluorescence released during PCR was measured by real-time PCR system and is directly proportional to the amount of the PCR product generated. The cycle number at which the fluorescence signal crosses a detection threshold is referred to as C_T and the difference of both C_T values within a sample (ΔC_T) is calculated ($\Delta C_T = C_{T\text{-FAM}} - C_{T\text{-VIC}}$). All samples were measured in duplicate using the mean for further analysis. For precise quantification of the ratio of methylated to unmethylated alleles, the ΔC_T value

Table 1
Sequences of primers and MGB Taqman® probes for QAMA.

Target genes	Amplified sequence location	Forward primers	Reverse primers	MGB Taqman® probes	
				Methylated	Unmethylated
BRCA1	17: 41278096 to 41278175[a]	5′-GCAGTTTGGGTAAGTAGTTTTGTAAG-3′	5′-TTCCCTACCCCAAACAAATT-3′	5′-VIC-ACTACGTCCCGCAAA-MGBNFQ-3′	5′-6FAM-ACTACATCCCCACAAAC-MGBNFQ-3′
BRCA2	13: 32889345 to 32889428[a]	5′-GTTGGAGTAAAAAGAAAGGGATGG-3′	5′-CCTTAAAAATCCCAAACCACCC-3′	5′-VIC-AAACCGCCCCTATAC-MGBNFQ-3′	5′-6FAM-AAAACCACCCCTATACC-MGBNFQ-3′

[a] Ensembl (GRCh37/hg19) assembly.