Lipid Deregulation in Women Carrying the BRCA Mutations: Non invasive evaluation by two-dimensional Spectroscopy

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Introduction

BRCA1 and BRCA2 genes belong to the tumor suppressor family and patients with these genes are at increased risk of developing breast cancer (1). A BRCA1 or BRCA2 mutation carrier has approximately a 3% risk of getting breast cancer before the age of 30. However, this risk increases to almost 50% when the patient reaches the age of 50 and becomes 50%-80% at the age of 70 (2). BRCA2 mutation carriers have been shown to survive longer than those carrying BRCA1 mutations. This difference has been attributed to increased ovarian deaths in BRCA1 mutation carriers (3). In this study, we apply in vivo two-dimensional 2D localized correlation spectroscopy (L-COSY) to look for a premalignant state in the breast tissues of apparently healthy women carrying the BRCA gene mutations and others with a family history. We propose the hypothesis that those with the BRCA gene mutations would have altered chemistry reflective of a preinvasive state.

Methods

Ten healthy, nine BRCA1, and fourteen BRCA2 subjects were recruited and consented according to local institutional ethics. Ultrasound scans were performed on all subjects. MRI experiments were performed on a 3T Skyra whole-body scanner (Siemens AG), using 70-cm diameter body coil for signal excitation and two-cavity breast coil for signal reception (RAPID Biomedical, Germany). Prior to the spectroscopy data being acquired, routine breast imaging was undertaken (T2w and dynamic contrast-enhanced imaging) with injection of a neutral-chelate (Omniscan, GE Healthcare, Germany). Contrast was given to all subjects except healthy volunteers. Five of the BRCA1 patients were examined by the L-COSY method before and after contrast to exclude the effect of contrast on the spectra. All patients and controls underwent ultrasound examination. The L-COSY sequence was applied with a $TE_{initial}$ of 30ms, TR 1.5 s, 8 averages per increment, bandwidth 2000 Hz, t1 increment 0.8 ms, vector size of 1024 points, RF offset frequency was set on 3.2ppm, and the number of increments was 64. The "WET" water suppression method (4) was applied. Processing was done as described elsewhere (5) and is described briefly here. Cross and diagonal peak volumes were measured by using the Felix software, with the $(CH_2)n$ diagonal peak at 1.30 ppm as the internal chemical shift reference. Statistical Analysis: Evaluation of statistical significance and variance was done by using Excel (Microsoft, 2010). The statistical significances of all assigned cross and diagonal peaks were calculated with the use of a *t* test (two-tailed, two-sample, unequal variance), and biomarker ratios with $P < 0.05$ were identified. The data used for the *t* test were considered to be normally distributed. Spectroscopic voxel was positioned on lower left outer quadrant midway between fibroglandular tissue and fat.

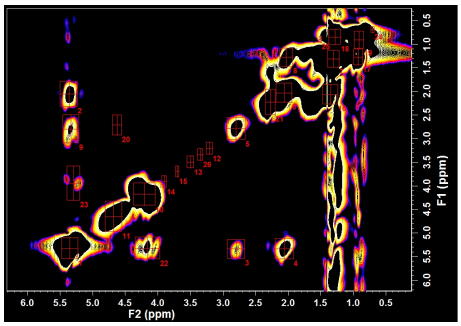

Figure 1. An L-COSY spectrum acquired from a BRCA2 subject.

Results and Discussion

No indication of breast abnormality was recorded by contrast enhanced MRI nor ultrasound examination for any group. Statistically significant ($P \leq 0.05$) changes were recorded in the lipid composition of BRCA1, BRCA2 gene mutation carriers when compared to healthy controls. A typical processed, peak-picked L-COSY spectrum of a BRCA2 gene carrier is shown in Figure 1. Many peak ratios produced statistically significant biological biomarkers for BRCA2+BRCA1, BRCA1, BRCA2 and healthy. We were able to distinguish between healthy, BRCA1 and BRCA2 cohorts using the current technique. Typical peak ratios with P-value less than 0.05 are listed in Table 1.

Pk ratio (F2,F1)PPM	Chemical species/Fragment	Pathology Average (Std Dev)	Pathology Average (Std Dev)	P-value
		BRCA2	**Healthy**	
3(2.7,5.3)a	-CH=C**H**-C**H2**-C**H**=CH-	0.04(0.01)	0.03(0.01)	0.03
		BRCA2+BRCA1	**Healthy**	
3(2.7,5.3)a	-C**H2**-C**H**=CH-	0.04(0.01)	0.03(0.01)	0.01
22(4.2,5.2)a	-CH-CH2 (backbone)	0.08(0.05)	0.05(0.01)	0.02
		BRCA1	**Healthy**	
14(3.9,3.9)c	GPC	0.05(0.02)	0.10(0.06)	0.04
		BRCA2	**BRCA1**	
13(3.5,3.5)b	Glycerol, Glycine, Ino, Cho	0.01(0.01)	0.00(0.00)	0.03

Table 1. Statistical analysis of COSY spectra showing average peak ratios for each class.
a: ratio to Pk1 (5.3,5.3) was calculated,
b: ratio to Pk10 (4.2,4.2) was calculated,
c: ratio to Pk5 (2.7,2.7) was calculated.

The following observations can be made from Table 1: (**1**) Combined BRCA group has altered levels of unsaturation compared with healthy (peak 22), (**2**) using 3.5ppm diagonal peak 13 (glycerol, glycine, Ino, Cho), BRCA1 and BRCA2 can be discriminated and (**3**) a higher ratio of glycerolphosphorcholine (GPC, 3.9ppm) to diallylic in healthy compared to BRCA1.

Limitations in the present study include small number per group, and that contrast is only given to BRCA carriers, even though it is established that Omniscan does not effect spectroscopic findings (6).

Conclusion

Deregulation of lipid metabolism is recorded in the in vivo breast tissue of women at high risk from BRCA1 and BRCA2 gene mutations. Lipid unsaturation alterations are established markers of malignant transformation (7). These results suggest that the 2D MR spectroscopy method can be used to screen high risk women by the addition of an 11 minute protocol to the standard MRI examination.

References:

1. Ont Health Technol Assess Ser 2010;10(3):1-55. 2. Veltman J, Mann R, Kok T, Obdeijn IM, Hoogerbrugge N, Blickman JG, Boetes C. European Radiology 2008;18(5):931-938. 3. Byrd L, Shenton A, Maher E, Woodward E, Belk R, Lim C, Lalloo F, Howell A, Jayson G, Evans D. Cancer Epidemiology, Biomarkers & Prev 2008;17(6):1535-1542. 4. Ogg RJ, Kingsley PB, Taylor JS. J Magn Reson Ser B 1994;104(1):1-10. 5. Ramadan S, Andronesi OC, Stanwell P, Lin A, Sorensen GA, Mountford C. Radiology 2011;259:540-549. 6. Lenkinski RE, Wang XE, Elian M, Goldberg SN. Magn Reson Med 2009;61(6):1286-1292. 7. MacKinnon WB, May GL, Mountford CE. Eur J Biochem 1992;205(2):827-839.