Analysis of chromosomal radiosensitivity of healthy BRCA2 mutation carriers and non-carriers in BRCA families with the G2 micronucleus assay

ANNELOT BAERT1, JULIE DEPUYDT1, TOM VAN MAERKEN2, BRUCE POPPE3, FRANSiska MALFAIT3, TIM VAN DAMME3, SYLVIA DE NOBELE3, GIANPAOLO PERLETTI1,4, KIM DE LEENEER3, KATHLEEN B.M. CLAES3* and ANNE VRAL1*

Departments of 1Basic Medical Sciences and 2Pediatrics and Medical Genetics, Ghent University, B-9000 Ghent; 3Center for Medical Genetics, Ghent University Hospital, B-9000 Ghent, Belgium; 4Department of Biotechnology and Life Sciences, University of Insubria, Busto Arsizio, I-21100 Varese, Italy

Received August 8, 2016; Accepted October 3, 2016

DOI: 10.3892/or.2017.5407

Abstract. Breast cancer risk drastically increases in individuals with a heterozygous germline BRCA1 or BRCA2 mutation, while it is estimated to equal the population risk for relatives without the familial mutation (non-carriers). The aim of the present study was to use a G2 phase-specific micronucleus assay to investigate whether lymphocytes of healthy BRCA2 mutation carriers are characterized by increased radiosensitivity compared to controls without a family history of breast/ovarian cancer and how this relates to healthy non-carrier relatives. BRCA2 is active in homologous recombination, a DNA damage repair pathway, specifically active in the late S/G2 phase of the cell cycle. We found a significantly increased radiosensitivity in a cohort of healthy BRCA2 mutation carriers compared to individuals without a familial history of breast cancer (P=0.046; Mann-Whitney U test). At the individual level, 50% of healthy BRCA2 mutation carriers showed a radiosensitive phenotype (radiosensitivity score of 1 or 2), whereas 83% of the controls showed no radiosensitivity (P=0.038; one-tailed Fisher’s exact test). An odds ratio of 5 (95% CI, 1.07-23.47) indicated an association between the BRCA2 mutation and radiosensitivity in healthy mutation carriers. We detected no increased radiosensitivity in the non-carrier relatives.

Introduction

BRCA1 and BRCA2 heterozygous mutation carriers have a strongly increased risk to develop breast cancer (BC) and ovarian cancer (OC). The lifetime risk to develop BC is 70-80% for BRCA1 mutation carriers and 50-60% for BRCA2 mutation carriers (1). For relatives who did not inherit the germline BRCA1/2 mutation segregating in the family (non-carrier relatives), the risk of BC occurrence is generally estimated to be as low as the risk assessed in the general population. This may imply that intensified BC detection screening, using, amongst others, mammography screening and MRI, as applied in individuals at high-risk is unnecessary in non-carriers (2-7). However, one study reported a 2-5-fold increase in BC occurrence in non-carriers of families with either BRCA1 or BRCA2 mutations (8). Another study reported a younger than expected age at diagnosis of BC for non-carriers, that was most evident in BRCA1 families (9). Moreover, the authors of these studies recommend targeted BC detection screening using for example mammography in non-carriers at a frequency comparable to the intensive BC screening performed in individuals at high-risk.

Both BRCA1 and BRCA2 are caretaker genes playing different roles in the repair of DNA double-strand breaks (DSB), induced by exposure to genotoxic agents such as ionizing radiation (IR). While BRCA1 has a more general function in the detection and signaling of a DSB and in the activation of the G2/M cell cycle checkpoint, BRCA2 exerts a specific function in the recruitment of RAD51 recombinase to the DSB site. This latter event is essential for the activation of the homologous recombination (HR) pathway, that relies on the undamaged sister chromatid as a template for resynthesis.
of the damaged strand. This occurs in the late S and G2 phase of the cell cycle and leads to error-free repair of DSB (1).

Knowing that both \textit{BRCA1} and \textit{BRCA2} are important in the repair of DSB, exposure of mutation carriers to IR, a potent inducer of DSB, for either diagnostic or therapeutic purposes appears to be counterintuitive, as mutation carriers may be more prone to develop radiation-induced BC (11).

Radiosensitivity of \textit{BRCA1} mutation carriers has previously been reported in the literature and was investigated and confirmed by our research group by means of the G2 micro-nucleus (MN) assay in combination with an evaluation of the G2/M checkpoint efficiency in peripheral blood lymphocytes of \textit{BRCA1} mutation carriers compared to healthy volunteers (12). However, the impact of IR on heterozygous cells of healthy \textit{BRCA2} mutation carriers remains to be elucidated.

To date, several cohort studies were able to prove a positive correlation between exposure to diagnostic X-rays and BC risk in \textit{BRCA2} mutation carriers (11,13,14). Others however, could not detect a similar correlation (15-18). Furthermore, Bernstein \textit{et al} detected no increased induction of contratetal BC upon exposure to radiotherapy in \textit{BRCA2} mutation carriers (19). Such discrepancies are likely due to differences in inclusion criteria, data acquisition and other issues of the studies. It is however difficult and unethical to design long-term unbiased studies to evaluate the relationship between \textit{BRCA2} mutations, the exposure to diagnostic or therapeutic radiation and BC risk. \textit{In vitro} chromosomal assays are effective tools to investigate radiosensitivity. Chromosomal radiosensitivity testing on lymphocytes from \textit{BRCA2} mutation carriers has been performed with techniques such as the G0 MN and the G2 assays for chromatid breaks, occasionally enhanced with a whole-chromosome painting FISH (20-25). However, for several of these studies, it was unclear whether the \textit{BRCA2} heterozygotes were healthy individuals or BC patients, which was previously broached by Baeyens \textit{et al} (20). Furthermore, differences in the experimental setup make comparisons between studies difficult (26). Despite these differences, all but one study was able to detect an elevated chromosomal radiosensitivity in BC patients with a \textit{BRCA2} mutation. However, no comparison was made with sporadic BC patients. The study of Baeyens \textit{et al} previously demonstrated enhanced radiosensitivity in both BC patients with a \textit{BRCA1}/2 mutation and sporadic BC patients, suggesting that the enhanced sensitivity may not be the result of the mutation (20). No univocal results were achieved for healthy \textit{BRCA2} mutation carriers. Radiosensitivity in non-carrier relatives has not been studied extensively, only one study reported no increased radiosensitivity measured with the G0 MN and G2 chromatid break assay in a small cohort (n=10) of relatives of both \textit{BRCA1} and \textit{BRCA2} families without the familial mutation when compared to a population cohort (20).

In the present study, we aimed to investigate chromosomal radiosensitivity in healthy \textit{BRCA2} mutation carriers by means of the G2 MN assay. We previously used this assay and confirmed radiosensitivity in healthy \textit{BRCA1} mutation carriers (n=18) compared to healthy controls without a family history of BC or OC (n=20) (12), and in an ataxia-telangiectasia patient and family members (27). In addition, we also included healthy relatives not carrying the familial germline \textit{BRCA1} or \textit{BRCA2} mutation in the present study. This cohort of non-carriers was included to evaluate radiosensitivity in individuals with a comparable genetic background, but without the familial \textit{BRCA1} or \textit{BRCA2} mutation.

\textbf{Materials and methods}

\textit{Sample collection.} Blood samples were collected from individuals consulting the Centre for Medical Genetics of the Ghent University Hospital (CMG; Ghent, Belgium), in the context of predictive testing for hereditary BC. Heparin blood samples were collected for the G2 MN assay. In addition, EDTA samples were collected for mutation analysis. We collected blood samples from 18 \textit{BRCA2} mutation carriers and 17 subjects from both \textit{BRCA1} (n=9) and \textit{BRCA2} (n=8) families not showing the familial mutation (non-carriers). None of the individuals selected for the present study had developed cancer at the time of the blood sample collection. We also selected 18 blood samples from a historical cohort of healthy volunteers without a personal or familial history of BC or OC for optimal age and gender match, to determine the normal distribution of MN yields in unafflicted individuals from the general population (12).

The present study was approved by the Ethics Committee of Ghent University Hospital (B67020111641 d.d. 20/09/2011) and all participants signed an informed consent.

\textit{Molecular analysis.} All healthy individuals selected for the present study had a family history of BC or OC and a mutation in either \textit{BRCA1} or \textit{BRCA2} was identified in each proband. All \textit{BRCA2} mutation carriers are heterozygous for an unequivocal deleterious mutation. This was confirmed by Sanger sequencing of the relevant amplicon. Sanger sequencing was performed on the ABI3730XL instrument using the BigDye® Terminator Cycle Sequencing kit (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions; sequences were analyzed using the SeqPilot software (JSI Medical Systems GmbH, Ettenheim, Germany).

Molecular analyses were not performed in healthy volunteers due to the absence of a personal or familial anamnesis for BC or OC.

\textit{The G2 MN assay.} The G2 MN assay was performed as previously described (12). In brief, heparinized blood was cultured in the presence of phytohaemagglutinin (PHA; 2% v/v; Gibco, Grand Island, NY, USA) to stimulate T-lymphocyte division. After 3 days, a population of cycling lymphocytes was obtained and the culture was irradiated with a 2 Gy dose of 60Co γ-rays. We opted to use a dose of 2 Gy as this is a well-accepted dose for chromosomal radiosensitivity testing in lymphocytes (20-22,24). Immediately after irradiation, cytochalasin B (cyto B; 6 μg/ml; Sigma-Aldrich, St. Louis, MO, USA) was added to all cultures, including a non-irradiated culture. Cyto B blocks the cytokinesis and allows the identification of first-division cells as a binucleated (BN) cell. After an incubation period of 8 h, all cultures were fixed with the sequential addition of KCl (75 mM), a solution of methanol, acetic acid and Ringer (4:1:5), and a combination of methanol and acetic acid (4:1) to pelleted cells. Finally, the cell suspension was concentrated and spread on slides. Slides were stained with 4',6-diamidino-2-phenylindole (DAPI) and
scanned with a Metafer 4 platform and MN search software (MetaSystems GmbH, Altlussheim, Germany). The automated image analysis system selects BN cells and determines the number of MN/BN cells. BN cells and MN selection are manually checked for false positives or false negatives. For each condition, 2 cultures were prepared and 2 slides/culture were analyzed. A minimum of 600 BN cells were scored/coded slide. To assess individual radiosensitivity, a radiosensitivity score (RS score) was determined. The mean and SD of the MN yield of the group of healthy volunteers (HV) was set as the cut-off value to determine the RS score of HV, BRCA2 mutation carriers and non-carrier relatives. An MN yield higher than the meanHV + 1SDHV was scored as 1, indicating a milder radiosensitive phenotype, whereas a result higher than the meanHV + 2SDHV was scored as 2, and indicated a more severe radiosensitive phenotype. When the individual value was lower than the meanHV + 1SDHV, a score of 0 was attributed to the tested subject.

**Statistical analysis.** Age and gender differences among the 3 groups were judged by means of a one-way ANOVA and Chi-square test, respectively. The median, interquartile range, average and standard deviation of micronuclei yields (number of MN/1,000 BN cells) were assessed in each group of subjects. Intergroup differences of MN yields between HV, BRCA2 mutation carriers and non-carrier relatives of BRCA1 and BRCA2 pedigrees were analyzed by the Mann-Whitney-Wilcoxon test. A one-tailed Fisher's exact test was performed to compare the unpaired and independent proportion of patients showing a radiosensitive phenotype, evaluated by RS scoring. For both assays a 5% α error was set as the limit for statistical significance. The odds ratio (OR) was calculated, based on the RS scores in healthy individuals and BRCA2 mutation carriers, to assess the association between the presence of a BRCA2 mutation and radiosensitivity according to the following formula:

\[
OR = \frac{\# \text{BRCA2 mutation carriers with an RS} \geq 0 \times \# \text{healthy volunteers with an RS} = 0}{\# \text{BRCA2 mutation carriers with an RS} = 0 \times \# \text{healthy volunteers with an RS} > 0}
\]

The 95% confidence interval (CI) was used as a proxy for significance. The VassarStats platform and the SPSS software (IBM, version 23) were used to perform statistical analysis.

**Results**

The mean age did not significantly differ for the HV (35.3 years), the BRCA2 mutation carriers (40.9 years) and the non-carrier relatives (40.0 years) (P=0.56; one-way ANOVA). In addition, no significant difference in gender distribution was observed for these 3 groups (68, 61 and 71% of the individuals were female, respectively) (P=0.84; Chi-square test). The number of spontaneously occurring micronuclei (MN yields in non-irradiated samples) was not significantly different among the 3 groups of enrolled subjects (Table I and Fig. 1).

Compared to HV without a family history of BC/OC, BRCA2 mutation carriers showed a significant increase in mean MN yields after exposure to 2 Gy IR (P=0.046; Mann-Whitney).

Conversely, the radiation-induced MN yields were similar in relatives who did not inherit the familial BRCA1/2 mutation and healthy BRCA2 mutation carriers; P<0.05 indicates a significant difference determined by Mann-Whitney U test. Error bars represent the standard error of the mean. BN, binucleated; MC, mutation carriers.

| Group data | 0 Gy | 2 Gy |
|------------|------|------|
| Healthy volunteers (HV) | Median: 12 | 56  |
| Intergroup range | 9.75 | 27.5 |
| Mean | 14.33 | 61.22 |
| SD | 8.85 | 21.73 |
| BRCA2 mutation carriers (MC) | Median: 14 | 74  |
| Intergroup range | 7.75 | 54.75 |
| Mean | 16.11 | 86.11 |
| SD | 6.91 | 41.87 |
| P-value vs. healthy volunteers (Mann-Whitney) | 0.177 | 0.046 |
| Relatives who did not inherit the familial BRCA1/2 mutation | Median | 16 | 69 |
| Intergroup range | 8 | 26 |
| Mean | 17.23 | 68.11 |
| SD | 7.74 | 22.30 |
| P-value vs. healthy volunteers (Mann-Whitney) | 0.116 | 0.400 |

SD standard deviation, MN micronucleus, BN binucleated cells.

![Figure 1. Mean G2 micronucleus (MN) yield. Mean MN yield for healthy volunteers, healthy relatives who did not inherit the familial BRCA1/2 mutation and healthy BRCA2 mutation carriers; *P<0.05 indicates a significant difference determined by Mann-Whitney U test. Error bars represent the standard error of the mean. BN, binucleated; MC, mutation carriers.](image-url)
Table II. Overview of median, interquartile range, mean and SD of the micronucleus yield (#MN/1,000 BN) for healthy relatives who did not inherit the familial germline BRCA1/2 mutation.

| Goup data       | 0 Gy | 2 Gy |
|-----------------|------|------|
| Median          | 16.44| 69.04|
| Interquartile range | 12   | 57   |
| Mean            | 18.11| 66.98|
| SD              | 6.88 | 27.45|

Table III) however shows some variation. An OR of 5 (95% CI, 1.07-23.46) for BRCA2 mutation carriers vs. HV, indicates a significant association between the presence of a BRCA2 mutation and radiosensitivity according to our criteria.

Discussion

Results of the G2 micronucleus (MN) assay performed after exposure to 2 Gy γ-rays showed a significantly increased radiosensitivity in healthy BRCA2 mutation carriers compared to healthy controls. Previous studies with a large number of different techniques were able to demonstrate enhanced radiosensitivity in BC patients with a BRCA2 mutation, however, no univocal results were achieved for healthy BRCA2 mutation carriers (20-25). Non-carrier relatives of either BRCA1 or BRCA2 families did not show an increased radiosensitive phenotype compared to the cohort of healthy volunteers, which is in agreement with the study of Baeyens et al (20). We previously performed the G2 MN assay in a group of 18 healthy BRCA1 mutation carriers, and found a significantly increased MN yield after exposure to 2 Gy γ-rays (12). These findings are analogous to the results of the present study, performed in healthy carriers of pathogenic BRCA2 mutations. Fig. 3 shows the integration of the data from healthy BRCA1 mutation carriers in the present study. The detection of an increased mean MN yield in both BRCA1 and BRCA2 mutation carriers after exposure to ionizing radiation can be explained by their mutual role in DNA double-strand break repair reviewed by Roy et al (1).

In our previous study we also analyzed the G2/M checkpoint activity by the addition of caffeine, an agent abrogating the G2/M checkpoint, to the irradiated cultures and demonstrated a significantly impaired checkpoint activation in BRCA1 mutation carriers compared to healthy volunteers (12). Analysis of the G2/M checkpoint activation in the current BRCA2 cohort did not reveal a significant difference (data not shown). This result is in agreement with the fact that BRCA2 is not activated in healthy carriers compared to healthy volunteers (12). Analysis of the G2/M checkpoint pathway active in the S and G2 phase of the cell cycle, is extensively reported in literature (1). The present study, focusing on radiosensitivity testing of lymphocytes in these phases of the cell cycle, showed an OR of 5 (95% CI, 1.07-23.47) for healthy individuals with a heterozygous BRCA2 mutation compared to healthy controls. This indicates a positive association between the presence of a BRCA2 mutation and radiosensitivity that could be attributed to deficient HR capacity in heterozygous cells.

Two independent research groups have reported a decreased DSB repair capacity in BRCA2 heterozygous cells.
Table III. Germline mutation, family ID, micronucleus yields (#MN/1,000 BN) and RS score for BRCA2 mutation carriers, relatives who did not inherit the familial mutation (non-carrier relatives) and healthy volunteers (numbering of the nucleotides according to RefSeq nr. NM_000059.3; A of ATG start codon=nucleotide +1).

| BRCA2 mutation carriers | Non-carrier relatives | Healthy volunteers |
|-------------------------|-----------------------|--------------------|
| **ID** | **Family ID** | **Mutation: nucleotide** | **Mutation: protein** | **0 Gy** | **2 Gy** | **RS score** | **ID** | **Family ID** | **Family gene** | **0 Gy** | **2 Gy** | **RS score** | **ID** | **0 Gy** | **2 Gy** | **RS score** |
| M2.01 | BR-32-2170 | c.658_659delGT | p.(Val220fs*4) | 14 | 119 | 2 | NM.06 | BR-32-0156 | BRCA2 | 17 | 51 | 0 | D01 | 19 | 83 | 1 |
| M2.02 | BR-32-1748 | c.1389_1390del | p.(Val464fs*3) | 15 | 91 | 1 | NM.17 | BR-32-0342 | BRCA1 | 8 | 29 | 0 | D12 | 10 | 52 | 0 |
| M2.03 | BR-32-1748 | c.1389_1390del | p.(Val464fs*3) | 19 | 83 | 1 | NM.01 | BR-32-0645 | BRCA1 | 20 | 63 | 0 | D13 | 7 | 47 | 0 |
| M2.04 | BR-32-1748 | c.1389_1390del | p.(Val464fs*3) | 12 | 58 | 0 | NM.10 | BR-32-1134 | BRCA1 | 14 | 74 | 0 | D15 | 12 | 55 | 0 |
| M2.05 | BR-32-1748 | c.1389_1390del | p.(Val464fs*3) | 16 | 56 | 0 | NM.13 | BR-32-1225 | BRCA1 | 18 | 43 | 0 | D16 | 17 | 44 | 0 |
| M2.06 | BR-32-1758 | c.1989del | p.(Phe663fs*5) | 12 | 163 | 2 | NM.12 | BR-32-1225 | BRCA1 | 11 | 57 | 0 | D17 | 7 | 58 | 0 |
| M2.07 | BR-32-0884 | c.4171del | p.(Glu1391fs*19) | 37 | 65 | 0 | NM.07 | BR-32-1444 | BRCA1 | 12 | 66 | 0 | D21 | 13 | 48 | 0 |
| M2.08 | BR-32-0884 | c.4171del | p.(Glu1391fs*19) | 20 | 90 | 1 | NM.08 | BR-32-1444 | BRCA1 | 12 | 78 | 0 | D04 | 12 | 40 | 0 |
| M2.09 | BR-32-1759 | c.4936_4939del | p.(Glu1646fs*23) | 20 | 65 | 0 | NM.02 | BR-32-1494 | BRCA1 | 24 | 87 | 1 | D05 | 6 | 30 | 0 |
| M2.10 | BR-32-1759 | c.4936_4939del | p.(Glu1646fs*23) | 18 | 53 | 0 | NM.16 | BR-32-1967 | BRCA1 | 29 | 125 | 2 | D06 | 15 | 74 | 0 |
| M2.11 | BR-32-0156 | c.6275_6276del | p.(Leu2092Profs*7) | 12 | 63 | 0 | NM.03 | BR-32-0884 | BRCA2 | 20 | 91 | 0 | D29 | 9 | 29 | 0 |
| M2.12 | BR-32-1565 | c.6275_6276del | p.(Leu2092Profs*7) | 8 | 44 | 0 | NM.04 | BR-32-0884 | BRCA2 | 16 | 73 | 0 | D30 | 30 | 109 | 2 |
| M2.13 | BR-32-1930 | c.6275_6276del | p.(Leu2092Profs*7) | 23 | 183 | 2 | NM.09 | BR-32-1748 | BRCA2 | 21 | 70 | 0 | D32 | 7 | 96 | 1 |
| M2.14 | BR-32-1930 | c.6275_6276del | p.(Leu2092Profs*7) | 12 | 86 | 1 | NM.11 | BR-32-1758 | BRCA2 | 8 | 45 | 0 | D31 | 26 | 73 | 0 |
| M2.15 | BR-32-1920 | c.8167G>C | p.(Asp2723His) | 10 | 118 | 2 | NM.05 | BR-32-1759 | BRCA2 | 38 | 85 | 1 | D35 | 37 | 76 | 0 |
| M2.16 | BR-32-1628 | c.8332_8487del | p.(Ile2778Lysfs*40) | 22 | 29 | 0 | NM.14 | BR-32-1759 | BRCA2 | 12 | 69 | 0 | D37 | 6 | 75 | 0 |
| M2.17 | BR-32-0937 | c.8904delC | p.(Val2968fs*4) | 10 | 131 | 2 | NM.15 | BR-32-2170 | BRCA2 | 13 | 52 | 0 | D38 | 17 | 52 | 0 |
| M2.18 | BR-32-0082 | c.9256+1G>C | r.9118_9256del; p.(Val3040Aspfs*18) | 10 | 53 | 0 | | | | | | | | |

Median: 14.00, 74.00; 16.00, 69.00; 12.00, 56.00
Interquartile range: 7.75, 54.75; 8.00, 26.00; 9.75, 27.50
Mean: 16.11, 86.11; 17.23, 68.11; 14.33, 61.22
SD: 6.91, 41.87; 7.74, 22.30; 8.85, 21.73

The splice site mutation present in M2.18 was previously described by Claes et al (39), where it was erroneously defined as IVS24G>A. SD standard deviation, MN micronucleus, BN binucleated cells, RS radiosensitivity.
Keimling et al. used an enhanced green fluorescent protein (EGFP)-based assay to report impaired HR capacity in lymphoblastoid cells with a BRCA2 monoallelic truncating frameshift mutation. The presence of a frameshift mutation was confirmed in a BRCA2-knockdown HeLa cell line (29). Arnold et al. demonstrated distinct defects in DNA DSB repair in lymphoblastoid cell lines (LCLs) from heterozygous BRCA2 mutation carriers through analysis of γ-H2AX repair kinetics (30). Although, the latter study did not focus on DNA repair by HR, it indicates a malfunction of DSB repair in LCLs from BRCA2 mutation carriers that could be attributed to diminished HR activity.

Most mutation carriers enrolled in the present study (n=17/18, 94%) had a mutation resulting in a premature termination codon (PTC). The presence of a PTC mutation is expected to activate nonsense-mediated decay of the gene transcript. Previous research from various groups including ours, demonstrated a reduction in mutant mRNA to approximately half of the WT mRNA levels in lymphocytes of individuals with a PTC mutation in BRCA1 (12,31,32). Arnold et al (30) detected a similar mutant mRNA reduction for BRCA2 mutations leading to a PTC. Furthermore, Arnold et al (30) and Keimling et al (29) report distinct reduced protein levels in LCLs from heterozygous BRCA2 mutation carriers, although quantitative analysis of this variation was not performed. Previously, haploinsufficiency has been suggested as the mechanism for hereditary BC development in BRCA1 and BRCA2 mutation carriers (33). In the present study, a higher than expected number of radiosensitive individuals in the BRCA2 mutation carriers indicates that haploinsufficiency may also be responsible for the radiosensitive phenotype in carriers of a mutation generating a PTC. In the present study, only one individual with a deleterious missense mutation was included. This substitution results in an amino acid change at position p.2723 and impairs protein functionality as shown by a homology-directed DNA break-repair functional assay (34). For this individual we obtained a high RS score of 2. Further research in larger patient cohorts with different types of mutations is needed to evaluate whether the type of mutation influences the radiosensitive phenotype or whether there are additional parameters determining this phenotype.

Results of the G2 MN assay showed no increased radiosensitivity in the group of non-carrier relatives of both BRCA1 and BRCA2 families compared to a group of healthy volunteers. Furthermore, only 24% of non-carriers showed an elevated radiosensitivity at the individual level (RS score 1 or 2). This was not significantly different from the fraction of healthy volunteers (17%) that was found to have an increased RS score. In addition, no difference was observed between non-carriers from BRCA1 (RS score, 0 in 7/9 investigated relatives) and BRCA2 families (RS score, 0 in 6/8 investigated relatives). However, we observed some variation within the different groups. We hypothesize that modifiers may play a role: indeed, selected SNPs in DNA-damage repair genes and other common variants have been associated with increased radiosensitivity (35-37) and increased BC risk (35,38). Further and larger studies are needed to evaluate the subtle influence of possible modifying factors on BC risk and radiosensitivity.

In conclusion, the present study demonstrated higher radiosensitivity in healthy BRCA2 mutation carriers compared to healthy volunteers by means of the G2 MN assay after exposure of peripheral blood lymphocytes to a dose of 2 Gy-γ-rays. No increased radiosensitivity was observed in non-carrier relatives of BRCA1 and BRCA2 families. When evaluating radiosensitivity at the individual level, a significantly higher proportion of BRCA2 mutation carriers (50%) showed a mild or more severe radiosensitivity compared to healthy volunteers (17%) and non-carriers (24%). Furthermore, an OR of 5 indicated a positive association between the BRCA2 mutation and an increased radiosensitivity in healthy mutation carriers. These results indicate that care should be taken when applying ionizing radiation for either diagnostic or therapeutic purposes in BRCA2 mutation carriers. However, a study including a larger population of subjects carrying different types of BRCA2 mutations and non-carriers, must be performed to further elucidate the effect of each single mutation on the radiosensitive phenotype and the influence of possible underlying factors.

Acknowledgements

The authors would like to thank all participants who donated a blood sample for the present study. They thank Céline De Brock, Brecht Crombez, Ilse Coene, Johanna Aernoudt, Toke Thiron, Greet De Smet and Leen Pieters for their technical assistance. Professor Thierens is thanked for the use of the irradiation facility. The present study was funded by the Belgian Foundation against Cancer (project 2012-216).

References

1. Roy R, Chun J and Powell SN: BRCA1 and BRCA2: Different roles in a common pathway of genome protection. Nat Rev Cancer 12: 68-78, 2011.
2. Bernholtz S, Laitman Y, Kaufman B, Shimon-Paluch S and Friedman E: Phenocopy breast cancer rates in Israeli BRCA1/BRCA2 mutation carrier families: Is the risk increased in non-carriers? Breast Cancer Res Treat 132: 669-673, 2012.
3. Domchek SM, Gaudet MM, Stopfer JE, Fleischuah M, Powers J, Kauff N, Offit K, Nathanson KL and Robson M: Breast cancer risks in individuals testing negative for a known family mutation in BRCA1 or BRCA2. Breast Cancer Res Treat 119: 409-414, 2010.
4. Harvey SL, Milne RL, McLachlan SA, Friedlander ML, Birch KE, Weideman P, Goldgar D, Hopper JL and Phillips KA; kConFab Investigators: Prospective study of breast cancer risk for mutation-negative women from breast cancer families. Breast Cancer Res Treat 130: 1057-1061, 2011.
5. Korde LA, Mueller CM, Louden JT, Struwing JP, Nichols K, Greene MH and Mai PL: No evidence of excess breast cancer risk among mutation-negative women from BRCA1 or BRCA2 mutation-positive families. Breast Cancer Res Treat 125: 169-173, 2011.
6. Kuriyan J, John EM, Johnston DA, Felberg A, West DW, Miron A, Andrusik IL, Hopper JL, Knight JA, et al: Breast cancer risk for noncarriers of family-specific BRCA1 and BRCA2 mutations: Findings from the Breast Cancer Family Registry. J Clin Oncol 29: 4505-4509, 2011.
7. Nielsen HR, John J, Krogh L, Nielsen M and Skytte AB: No evidence of increased breast cancer risk for proven noncarriers from BRCA1 and BRCA2 families. Fam Cancer 15: 523-528, 2016.
8. Smith A, Moran A, Boyd MC, Bulman M, Shenton A, Smith L, Iddon R, Woodward ER, Laloo L, Maher ER, et al: Phenocopies in BRCA1 and BRCA2 families: Evidence for modifier genes and implications for screening. J Med Genet 44: 10-15, 2007.
9. Vas JR, de Bock GH, Teixeira N, van der Kolk DM, Jansen L, Mourits MJ, Eeckhout JC: Proven non-carriers in BRCA families with a breast cancer case at an earlier age of onset of breast cancer. Eur J Cancer 49: 2101-2106, 2013.
10. Evans DGR, Ingham SL, Buchan I, Woodward ER, Byers H, Howell A, Maher ER, Newman WG and Laloo L: Increased rate of phenocopies in all age groups in BRCA1/BRCA2 mutation carriers in the International BRCA1/BRCA2 carrier cohort study: A report from the EMBRACE, GENEPSO, GEO-HEBON, and IBCCS Collaborators’ Group. J Clin Oncol 24: 3361-3366, 2006.
11. Baert A, Depuydt J, Van Maeerken T, Poppe B, Mulfiai F, Storm K, van den Ende J, Van Damme T, De Nobele S, Perletti G, et al: Increased chromosomal radiosensitivity in asymptomatic carriers of a heterozygous BRCA1 mutation. Breast Cancer Res 18: 52, 2016.
12. Andrieu N, Easton DF, Keskiniemi A, Cardis E, Nougé C, Gauthier-Villars M, Lasset C, Fricker JP, Peock S, et al; GENEPSO; BRCA1/2; GEO-HEBON: Exposure to diagnostic radiation and risk of breast cancer among carriers of BRCA1/2 mutations: Retrospective cohort study (GENE-RAD-RISK). BMJ 345: e5660, 2012.
13. Pippe A, Andrieu N, Easton DF, Keski-Muotia A, Cardis E, Nougé C, Gauthier-Villars M, Lasset C, Fricker JP, Peock S, et al; GENEPSO; BRCA1/2; GEO-HEBON: Exposure to diagnostic radiation and risk of breast cancer among carriers of BRCA1/2 mutations in the international BRCA1/2 carrier cohort study: A report from the EMBRACE, GENEPSO, GEO-HEBON, and IBCCS Collaborators’ Group. J Clin Oncol 24: 3361-3366, 2006.
14. Lecarpentier J, Nougé C, Mouret-Fourme E, Stoppa-Lyonnet D, Lasset C, Caron O, Fricker JP, Gladieff L, Faireve L, Sobol H, et al; GENEPSO: Variation in breast cancer risk with mutation position, smoking, alcohol, and chest X-ray history, in the French National BRCA1/2 carrier cohort (GENEPSO). Breast Cancer Res Treat 130: 927-938, 2011.
15. Narod SA, Lubinski J, Ghadirian P, Lynch HT, Moller A, Pankratz VS, Singh N, Thompson J, Erding CA, et al; Variant ataxia telangiectasia: Clinical and molecular findings associated with breast cancer risk/susceptibility. PLoS One 9: e112354, 2014.
16. Bernstein JL, Thomas DC, Shore RE, Robson M, Boice JD Jr, Stovall M, Andersson M, Bernstein L, Malone KE, Reiner AS, et al; WE CARE Study Collaborative Group: Contralateral breast cancer after radiotherapy among BRCA1 and BRCA2 mutation carriers: A WE CARE study report. Eur J Cancer 49: 2979-2985, 2013.
17. Bailey J, Thierens H, Claes K, Poppe B, de Ridder L and Vral A: Chromosomal radiosensitivity in BRCA1 and BRCA2 mutation carriers. Int J Radiat Biol 80: 745-756, 2004.
18. Gutiérrez-Enríquez JD, Rojo S, Ramon Y Cajal T, Alonso C, Corral A, Carrasco P, Cornet M, Sanz J, Ribas M, Baiget M and Diez O: Ionizing radiation and mitomycin-induced micronuclei in lymphocytes of BRCA1 or BRCA2 mutation carriers. Breast Cancer Res Treat 136: 617-622, 2011.
19. Trenz K, Rothfuss A, Schütz P and Speit G: Mutagen sensitivity of peripheral blood from carrying a BRCA1 or BRCA2 mutation. Mutat Res 500: 89-96, 2002.
20. Beroukas E, Pandis N, Giannakoulakos K, Rizou E, Beroukas K, Giatromanolaki A and Koukourakis M: Increased chromosomal radiosensitivity in women carrying BRCA1/BRCA2 mutations assessed with the G2 assay. Int J Radiat Oncol Biol Phys 76: 1199-1205, 2010.
21. Becker AA, Graeser MK, Landwehr C, Hilger T, Baus W, Wappenschmidt B, Meindl A, Weber RG and Schmutzler RK: A 24-color metaphase-based radiation assay discriminates heterozygous BRCA2 mutation carriers from controls by chromosomal radiosensitivity. Breast Cancer Res Treat 135: 267-276, 2012.
22. Bolognesi C, Bruzzi P, Gismondi V, Volpi S, Viassolo V, Pedemonte S and Varesco L: Clinical application of micro-nucleus test: A case-control study on the prediction of breast cancer risk/susceptibility. PLoS One 9: e112354, 2014.
23. Cardinale F, Bruzzi P and Botulin P: Role of micronucleus test in predicting breast cancer susceptibility: A systematic review and meta-analysis. Br J Cancer 106: 780-790, 2012.
24. Claes K, Depuydt J, Taylor AMR, Last J, Baert A, Schietecatte P, Vandersickel V, Poppe B, De Leeener K, D’Hooghe M, et al: Variant ataxia telangiectasia: Clinical and molecular findings and qualitative evaluation of radiosensitive phenotypes in a patient and relatives. Neuromolecular Med 15: 447-457, 2013.
25. Menzel T, Nähse-Kumpf V, Kousholt AN, Klein DK, Lund-Andersen C, Lees M, Johansen JV, Syljäsen RG and Sørensen CS: A genetic screen identifies BRCA2 and PALB2 as key regulators of G2 checkpoint maintenance. EMBO Rep 12: 705-712, 2011.
26. Keimling M, Volpic M, Csernok A, Wieland B, Dörker T and Wiesmüller L: Functional characterization connects individual patient mutations in ataxia telangiectasia mutated (ATM) with dysfunction of specific DNA double-strand break repair mechanisms. FASEB J 25: 3849-3860, 2011.
27. Arnold K, Kim MK, Freker K, Edler L, Saveleva L, Schmezer P and Wiedemeyer R: Lower level of BRCA2 protein in heterozygous mutation carriers is correlated with an increase in DNA double strand breaks and an impaired DSBR repair. Cancer Res 73: 90-100, 2006.
28. Perriñ-Vidóz L, Sinilnikova OM, Stoppa-Lyonnet D, Lenoir GM and Mazoyer S: The nonsense-mediated mRNA decay pathway triggers degradation of most BRCA1 mRNAs bearing premature termination codons. Hum Mol Genet 11: 2849-2860, 2002.
29. Anczuków O, Ware MD, Buissso N, Zetoune AB, Stoppa-Lyonnet D, Sinilnikova OM and Mazoyer S: Does the nonsense-mediated mRNA decay mechanism prevent the synthesis of truncated BRCA1, CHK2, and p53 proteins? Hum Mutat 29: 65-73, 2008.
30. Berger AH, Knudson AG and Pandolfi PP: A continuum model for tumour suppression. Nature 476: 163-169, 2011.
31. Guidugli L, Pankratz VS, Singh N, Thompson J, Erding CA, Engel C, Schmutzler R, Domchek S, Nathanson K, Radice P, et al: A classification model for BRCA2 DNA binding domain missense variants based on homology-directed repair activity. Cancer Res 73: 265-275, 2013.
32. Wappenschmidt B, Meindl A, Weber RG and Schmutzler RK: A 24-color metaphase-based radiation assay discriminates heterozygous BRCA2 mutation carriers from controls by chromosomal radiosensitivity. Breast Cancer Res Treat 135: 267-276, 2012.
33. Wiesmüller L: Functional characterization connects individual patient mutations in ataxia telangiectasia mutated (ATM) with dysfunction of specific DNA double-strand break repair mechanisms. FASEB J 25: 3849-3860, 2011.
34. Lyonnet D, Sinilnikova OM and Mazoyer S: The nonsense-mediated mRNA decay pathway triggers degradation of most BRCA1 mRNAs bearing premature termination codons. Hum Mol Genet 11: 2849-2860, 2002.
35. Allocco L, Felberg A, West DW, Miron A, Knight JA, et al; Kathleen Cunningham Foundation Consortium for Research into Familial Breast Cancer (kConFab); Whitemore AS: Diagnostic chest X-rays on the risk of breast cancer among BRCA1 and BRCA2 mutation carriers. Cancer Epidemiol Biomarkers Prev 22: 1547-1556, 2013.
36. Huo S, Shi Y, Zhou H, Zhang W and Wang H: Radioimmunotherapy helps to achieve personalized therapy by evaluating patient responses to radiation treatment. Carcinogenesis 36: 307-317, 2015.
37. Popanda O, Marquardt JU, Chang-Claude J and Schmezer P: Genetic variation in normal tissue toxicity induced by ionizing radiation. Mutat Res 667: 58-69, 2009.
38. Antoniou AC, Kuchenbaecker KB, Soucy P, Beesley J, Chen X, McGuffog L, Lee A, Barrowdale D, Healey S, Sinilnikova OM, et al; CIMBA, SWE-BRCA; HEBON, ÉMIRACE; GEMO Collaborators Study; kConFab Investigators: Common variants at 12p11, 12q24, 9p21, 9q31.2 and in ZNF365 are associated with breast cancer risk for BRCA1 and/or BRCA2 mutation carriers. Breast Cancer Res 14: R33, 2012.

39. Claes K, Poppe B, Machackova E, Coene I, Foretova L, De Paepe A and Messiaen L: Differentiating pathogenic mutations from polymorphic alterations in the splice sites of BRCA1 and BRCA2. Genes Chromosomes Cancer 37: 314-320, 2003.