BRCA2 Mutations and Triple-Negative Breast Cancer

Peter Meyer1*, Katharina Landgraf1, Bernhard Högel2, Wolfgang Eiermann3, Beyhan Ataseven3

1 Institute of Medical Genetics, University Hospital, Rostock, Germany, 2 Institute of Pathology, Red Cross Hospital, Munich, Germany, 3 Frauenklinik vom Roten Kreuz, Munich, Germany

Abstract

Recently, BRCA1 germline mutations were found in a high proportion (14–34%) of patients with triple-negative breast cancer (TNBC). BRCA2 was either not analyzed or showed much lower mutation frequencies. Therefore, we screened a group of TNBC patients (n = 30) of white European descent for mutations in BRCA2 as well as in BRCA1. Cases were unselected for age of disease-onset (median age at breast cancer diagnosis was 58 years, ranging from 37 to 74 years), family history of cancer and BRCA1 and BRCA2 mutation status. Half of the patients (15/30) showed a family history of breast and/or ovarian cancer. A high frequency of deleterious germline mutations was observed in BRCA2 (5/30; 16.7%), and only one case showed a BRCA1 mutation (3.3%). Although the study group was small, these results point to BRCA2 mutations being important in TNBC.

Introduction

Recently, triple-negative breast cancer (TNBC) has been classified as a breast cancer subgroup that is negative for estrogen and progesterone receptors (ER/PR) and receptor 2 of human epidermal growth factor (HER2). TNBC accounts for approximately 15% of all breast cancer cases and seems to be closely related to basal-like breast cancer, which has an expression profile similar to that of normal basal-myoepithelial breast tissue. Approximately 80% of TNBC cases show a basal-like phenotype, and the majority of basal-like breast cancers can be classified as TNBC. Close to 75% of BRCA1-associated breast cancers is either TNBC, basal-like breast cancer or both. TNBC patients have a relatively poor outcome and cannot be treated with endocrine therapy or therapies targeted to HER2 due to the lack of related receptors [1].

Recent reports have focused on BRCA1 germline mutations in TNBC cases. In a Canadian TNBC cohort (n = 54) with an age of onset before 41 years and no familial breast cancer aggregation, five cases with mutations of BRCA1 (9%) and only one case with a mutation of BRCA2 (2%) were detected. The entire coding sequence of BRCA1 and the large exons 10 and 11 of BRCA2 were analyzed [2]. Of 73 TNBC cases from the United Kingdom (UK) studied, 16 (22%) had BRCA1 mutations and none had BRCA2 mutations when fully screened in both genes. Forty-three of these cases were from patients younger than 41 years of age at diagnosis and with no familial aggregation of breast cancer. An additional 30 cases were unselected for family history of breast cancer because they were younger than 31 years old at onset of the disease [3]. In 77 TNBC cases from the United States that were not selected for age or familial breast cancer occurrence, the mutation frequencies were 15.6% for BRCA1 (n = 12) and 3.9% for BRCA2 (n = 3). The entire coding sequence and the exon-intron boundaries were screened in both genes [4]. Finally, a cohort of 64 Ashkenazi Jewish TNBC patients unselected for age of onset and familial aggregation of breast cancer was screened for mutations in BRCA1 and BRCA2. In all Ashkenazi Jewish breast cancer cases, approximately 10% of patients carry one of three different founder mutations in BRCA1 and BRCA2. In the investigated Ashkenazi Jewish TNBC cohort (n = 64), 19 BRCA1 (29.7%) and 6 BRCA2 (9.4%) mutations could be detected when screening for the three founder mutations [5]. Given that the recent reports on TNBC cases describe either no or low frequencies of BRCA2 mutations [3,4], or refer to very special inbred populations with high founder mutation frequencies [5], or report on incomplete BRCA2 mutation screening approaches [2], we aimed to investigate the BRCA2 mutation frequency in a TNBC cohort of a Southern German population unselected for age of onset and familial aggregation of cancer. If there was a relevant mutation rate in such cases, TNBC would meet the clinical criteria for mutation screening in BRCA2 and should be added to the current guidelines.

Methods

Ethics statement

All selected patients underwent genetic counseling and gave their written informed consent for genetic testing. The study protocol was approved by the Ethics Committee of the State of Salzburg (Austria).

Study population

In a monocentric approach executed in Munich, Germany, newly diagnosed cases of individuals with TNBC diagnosed between 2005 and 2010 were selected from the Pathology Unit. Histological samples were classified as TNBC when the following criteria were met: less than 1% of cells demonstrated nuclear staining for estrogen and progesterone receptors, and immuno-
| DNA | BRCA1 Category | BRCA1 Mutation | BRCA2 Category | BRCA2 Mutation | Age at Diagnosis (Age) | Second Tumor (Age) | Family History of Cancer (Age) | Criteria for Mutation Screening* |
|-----|----------------|----------------|----------------|----------------|-----------------------|-------------------|-----------------------------|----------------------------------|
| 1110 | LCS            | p.R496H        | PM; RP (TNBC)  | c.1029delA; p.T1915M | 69 0                 | 6 x BC ms          | yes                         |                                  |
| 1129 | -              | -              | PrPM           | p.W2626C       | 61 0                 | 4x lung cancer ms, grandmother ms BC (61), 4 add. cancers ms | no                            |                                  |
| 1153 | PM             | c.5266dupC     | -              | -              | 43 BC (48)           | mother BC (60)     | yes                         |                                  |
| 1156 | -              | -              | PM             | p.51882X       | 37 0                 | 0                 | no                          |                                  |
| 1186 | -              | -              | PM             | c.476-1G>A     | 56 0                 | sister BC (52), mother OvCa (63) | yes                     |                                  |
| 1245 | -              | -              | PM             | c.6444dupT     | 38 BC (43)           | mother OvCa (60)   | yes                         |                                  |
|      |                |                |                |                |                      |                   |                             | Median 50                       |
| 1102 | -              | -              | LCS            | p.A2951T       | 38 0                 | 0                 | no                          |                                  |
| 1106 | -              | -              | RP (TNBC)      | p.T1915M       | 48 BC (56)           | mother BC (45)     | yes                         |                                  |
| 1108 | -              | -              | -              | -              | 71 0                 | 0                 | no                          |                                  |
| 1109 | -              | -              | -              | -              | 63 skin              | sister BC (58)     | no                          |                                  |
| 1111 | -              | -              | -              | -              | 64 0                 | aunt ms BC (68), aunt ms BC (50) | yes                     |                                  |
| 1115 | LCS            | p.M1652I       | -              | -              | 59 0                 | 0                 | no                          |                                  |
| 1117 | -              | -              | -              | -              | 57 basalioma (56)    | 0                 | no                          |                                  |
| 1118 | -              | -              | -              | -              | 51 uterus (20; cis)  | sister BC (50)     | yes                         |                                  |
| 1130 | -              | -              | -              | -              | 70 0                 | mother BC (41)     | yes                         |                                  |
| 1132 | -              | -              | -              | -              | 43 0                 | aunt fs BC (42+50)  | yes                         |                                  |
| 1135 | -              | -              | -              | -              | 48 0                 | 0                 | no                          |                                  |
| 1139 | LCS            | p.M1652I       | -              | -              | 43 BC (57), basalioma (58)* | yes                  |                             |                                  |
| 1141 | -              | -              | -              | -              | 74 0                 | 0                 | no                          |                                  |
| 1145 | -              | -              | US             | c.68-7T>A      | 57 0                 | 0                 | no                          |                                  |
| 1178 | -              | -              | RP (TNBC)      | p.T1915M       | 70 0                 | aunt ms OvCa       | yes                         |                                  |
| 1180 | -              | -              | US             | p.F1524V       | 49 0                 | 0                 | no                          |                                  |
| 1182 | -              | -              | -              | -              | 63 0                 | 3 x BC ms, 2x lung cancer ms | yes                     |                                  |
| 1201 | -              | -              | RP (TNBC)      | p.T1915M       | 63 0                 | sister of grandmother ms BC | no                      |                                  |
| 1217 | -              | -              | -              | -              | 50 0                 | 0                 | no                          |                                  |
| 1219 | -              | -              | -              | -              | 70 0                 | cousin ms BC (40)   | yes                         |                                  |
| 1232 | LCS            | p.M1652I       | -              | -              | 63 0                 | niece bilateral BC (32), aunt ms BC (42) | yes                     |                                  |
| 1244 | -              | -              | -              | -              | 66 0                 | aunt fs BC          | no                          |                                  |
| 1252 | LCS            | p.M1652I       | -              | -              | 68 0                 | sister BC (66), grandmother fs BC (50) | yes                     |                                  |
| 1259 | -              | -              | -              | -              | 56 0                 | 0                 | no                          |                                  |

Median 61 11/24 (46%)
histrochemical staining for HER2 showing a 0, 1-fold, or a 2-fold positive score and a FISH ratio (HER2 gene signals to chromosome 17 signals) of less than 1.8 according to the relevant ASCO and CAP guidelines [6]. No further selection criteria were applied. For immunohistochemistry (IHC), we used IgG1 mouse antibodies for estrogen (clone 6F11) and for progesterone receptors (clone 16; both antibodies were purchased from DCS Innovative Diagnostik-Systeme, Dr. Christian Sartori GmbH & Co. KG, Hamburg, Germany). For HER2-IHC we used polyclonal rabbit antibodies from clone A0485 (HercepTM-Test; Dako Deutschland GmbH, Hamburg, Germany).

Genotyping

DNA extraction from whole blood samples (EDTA) was performed according to standard protocols. To amplify exons and exon-intron boundaries of BRCA1 and BRCA2, we used primer pairs and polymerase chain reaction (PCR) protocols published elsewhere [7]. PCR products were sequenced using BigDye® Terminator Cycle Sequencing Kits and 3730xl DNA Analyzers with 96 capillary arrays according to the manufacturer’s protocols (Applied Biosystems, Foster City, CA 94404 USA). To exclude deletions and duplications of one or more exons, we performed Multiplex Ligation-dependent Probe Amplification (MLPA) of both genes according to the company’s technical protocols (MRC-Holland, Amsterdam, the Netherlands). Mutations and variants were classified according to the kConFab classification scheme [8].

Clinical criteria for mutation screening in BRCA1 and BRCA2

In Germany, patients with a 10% mutation probability are eligible for mutation screening in BRCA1 and BRCA2. The following clinical criteria were evaluated in approximately 1,000 unrelated cases in a German-wide study:

- Two women with breast cancer, with one of them ≤50 years, or three women with breast cancer, independent of age.
- One woman with breast and one woman with ovarian cancer, independent of age, or one woman with breast and ovarian cancer, independent of age, or two women with ovarian cancers, independent of age.
- One male breast cancer and one woman with breast or ovarian cancer.
- One woman with breast cancer ≤35 years.
- One woman with bilateral breast cancer or two primary breast cancers, with first tumour diagnosed before ≤50 years [9,10].

Results

Characteristics of investigated patients are presented in Table 1. Six out of 30 TNBC patients (20%) carried a deleterious mutation – one (1/30; 3.3%) in BRCA1 and five (5/30; 16.7%) in BRCA2. We detected four known deleterious frame-shift mutations (BRCA1: c.5266dupC (old nomenclature is “5382insC”) which is one of the most common deleterious BRCA1 mutations [11]; BRCA2: c.1029delA [12], p.S1882X [13], c.6444dupT accession number 3790 in BIC database [http://research.nhgri.nih.gov]) and one unknown splice-site mutation (BRCA2: c.476-1G>A) with a clear predictive deleterious effect. One missense mutation in BRCA2 (p.W2626C) was classified as “predictive deleterious” in prior literature [13].

In four cases, we detected c.4956G>A in BRCA1 leading to an exchange of methionine for isoleucine at amino acid position
1,652 of the protein (p.M1652I; 4/30; 13.3%). Variant c.5744C>T causes a point mutation of threonine to methionine at amino acid position 1,915 of BRCA2 and was detected in four cases (p.T1915M; 4/30; 13.3%). Within mutation carriers, two out of six cases (33.3%) did not meet the German clinical criteria for BRCA1 and BRCA2 testing. From all TNBC cases, 15 out of 30 (50%) showed an early age of onset or a familial aggregation which are German clinical criteria for mutational screening of BRCA1 and BRCA2. The median age at diagnosis of the total study group was 58 years (n = 30), for mutations carriers 50 years (n = 6), and for the remaining patients (n = 24) not showing deleterious mutations 61 years.

**Discussion**

The aim of this study was to investigate the role of BRCA2 germline mutations in triple-negative breast cancer (TNBC). We detected 6 deleterious BRCA1 and BRCA2 mutations in 30 TNBC patients (20%). The total mutation frequency for both genes is comparable with those described in recent studies [5,3,4,2]. In contrast, we found a high frequency of BRCA2 germline mutations in our TNBC study group: four cases with previously published deleterious variants, and one splice-site mutation which was not published before (16.7%). The low rate of BRCA2 mutations found in a Canadian sample (1/34 = 2%) was probably the result of assay design given that only exons 10 and 11 of BRCA2 were tested. Additionally, only TNBC cases with an age of disease onset of less than 41 years were selected for the study [2]. Our patient group was not selected for age of disease onset; the average age was 57 years, with a median age of 58 years. This age difference between the two study groups could also explain the inconsistent frequencies of BRCA2 mutations. No BRCA2 mutations were detected in 73 TNBC cases from the UK, BRCA1 and BRCA2 genes were fully screened, but patients were diagnosed with TNBC before age 41 (n = 49) and before age 30 (n = 30). Cases were selected for the UK study only if no familial aggregation was reported [3]. In contrast, our TNBC group was not selected for familial tumour aggregation or age of onset; these differences in inclusion criteria may explain the different BRCA2 mutation frequencies. Interestingly, a similar study of 77 TNBC cases from the United States where cases were unscreened for age of onset (average was 51 years) revealed a much lower BRCA2 mutation rate (3.9% vs. 16.7% in our study) [4].

Recently, data of a multi-national approach containing approximately 7,000 BRCA1 and BRCA2 mutation carriers (BRCA1: n = 4,325; BRCA2: n = 2,568) showed that the proportion of TNBC increased with age at diagnosis in BRCA2 mutation carriers, while it decreased in BRCA1 mutation carriers [14]. These findings are in accordance with our results since our patient group tended to be older than those reporting lower BRCA2 and higher BRCA1 mutation rates in TNBC [5,3,4,2].

In four cases, we detected c.4956G>A in BRCA1 (p.M1652I; 4/30; 13.3%). Analysis with protein prediction software showed some minor effect [15], so we decided to classify this variant as reaching, at most, “low clinical significance” (LCS; table 1). Variant c.5744C>T was detected in four cases (p.T1915M; 4/30; 13.3%). Methionine at amino acid position 1,915 of BRCA2 (p.M1652I) was less frequent (OR 0.39, 95% CI 0.19 – 0.82, p = 0.013) in estrogen receptor-positive patients of a study cohort of 117 sporadic breast cancer cases from Poland [16]. We therefore classified this variant as “risk-conferring polymorphism” (table 1). The two variants (c.4956G>A in BRCA1 and c.5744C>T in BRCA2) could therefore be investigated in much larger association studies to evaluate their impact in TNBC tumorigenesis.

We identified deleterious BRCA2 mutations in two patients not meeting the current German clinical criteria for BRCA1 and BRCA2 mutation screening. In our cohort of TNBC patients unselected for age at diagnosis and cancer family history, 15 cases (15/30; 50%) did not meet those criteria. The mutation rate in this subgroup was 13% (2/15). The main requirement for the German clinical criteria for BRCA1 and BRCA2 mutation testing was defined as being at least a 10% priori probability to carry either a BRCA1 or BRCA2 mutation [9,10]. If larger studies confirm our findings, TNBC therefore may be included within clinical criteria for BRCA1 and BRCA2 mutation screening, and additional cases may be assessed not only if they present with early-onset of the disease or have a familial aggregation of breast and/or ovarian cancer. BRCA1 and BRCA2 mutation carriers are frequently diagnosed with triple-negative breast cancer [14]. Nevertheless, the German clinical selection criteria do not recommend BRCA1 and BRCA2 mutation screening in TNBC. In contrast, recent guidelines published by the National Comprehensive Cancer Network in the United States are less stringent. For instance, TNBC diagnosed before age 60, and breast cancer cases with a negative family history for cancer younger than 45 years should be tested according to these guidelines. In comparison, the German guidelines recommend testing single cases only if the diagnosis was made before age 35 [9,10,17].

In summary, we found a 20% germline mutation rate (6/30 cases) in BRCA1 and BRCA2 genes in patients diagnosed with TNBC from Southern Germany and unselected for age of disease onset and familial aggregation of this malignant disease. In contrast to recent reports, an unexpected and high mutation frequency was seen in BRCA2 (5/30; 16.7%) suggesting that this gene may play an important role in the development of TNBC when mutated. Our data suggest that Southern German patients diagnosed with TNBC may qualify for BRCA1 and BRCA2 mutation screening. Larger patient cohorts have to be investigated to validate our findings.

**Acknowledgments**

We thank our patients and their families for their kind cooperation.

**Author Contributions**

Conceived and designed the experiments: PM KL BH WE BA. Performed the experiments: PM KL BH WE BA. Analyzed the data: PM KL BH WE BA. Contributed reagents/materials/analysis tools: PM KL BH WE BA. Wrote the paper: PM.

**References**

1. Foulkes WD, Smith IE, Reis-Filho JS (2010) Triple-negative breast cancer. N. Engl. J. Med 363(20): 1938-1948.
2. Young SR, Pisanski RT, Donenberg T, Shapiro C, Hammond LS et al (2009) Incidence and outcome of BRCA mutations in unselected patients with triple receptor-negative breast cancer. BMC Cancer 9: 86.
3. Evans DG, Howell A, Ward D, Laloo F, Jones JL et al (2011) Prevalence of BRCA1 and BRCA2 mutations in triple negative breast cancer. J. Med. Genet 48(8): 520-522.
4. Gonzalez-Angulo AM, Timms KM, Liu S, Chon H, Littion JK et al (2011) BRCA Mutations and Triple-Negative Breast Cancer. Breast Cancer Res. Treat 129(1): 185–190.
5. Comen E, Davids M, Kirchhoff T, Hudis C, Offit K et al (2011) Relative contributions of BRCA1 and BRCA2 mutations to “triple-negative” breast cancer in Ashkenazi women. Breast Cancer Res. Treat 129(1): 185-190.
7. Meyer P, Voigtlaender T, Bartram CR, Klaes R (2003) Twenty-three novel BRCA1 and BRCA2 sequence alterations in breast and/or ovarian cancer families in Southern Germany. Hum. Mutat 22(3): 259 p.

8. kConFab Classification of BRCA1 and BRCA2 Mutations. Available: http://www.kconfab.org/Progress/Classification.shtml.

9. Bauerfeind I (2011) Mammakarzinome. Diagnostik [Empfehlungen zur, ed. Therapie und Nachsorge]. München [i.e.] Germering [u.a.]: Zuckschwerdt. XII, 340 S.

10. Gadzicki D, Evans DG, Harris H, Julian-Reynier C, Nippert I et al (2011) Genetic testing for familial/hereditary breast cancer—comparison of guidelines and recommendations from the UK, France, the Netherlands and Germany. J Community Genet 2(2): 53–69.

11. Antoniou AC, Pharoah PDP, Narod S, Risch HA, Eyfjord JE et al (2005) Breast and ovarian cancer risks to carriers of the BRCA1 5382insC and 185delAG and BRCA2 6174delT mutations: a combined analysis of 22 population based studies. J Med. Genet 42(7): 602–603.

12. Risch HA, McLaughlin JR, Cole DEC, Rosen B, Bradley L et al (2006) Population BRCA1 and BRCA2 mutation frequencies and cancer penetrances: a kin-cohort study in Ontario, Canada. J Natl. Cancer Inst 98(23): 1694–1706.

13. Borg A, Haile RW, Malone KE, Caplanu M, Diep A et al (2010) Characterization of BRCA1 and BRCA2 deleterious mutations and variants of unknown clinical significance in unilateral and bilateral breast cancer: the WECARE study. Hum. Mutat 31(3): E1200–40.

14. Mavaddat N, Barrowdale D, Andriole LL, Domchek SM, Eccles D et al (2012) Pathology of breast and ovarian cancers among BRCA1 and BRCA2 mutation carriers: results from the Consortium of Investigators of Modifiers of BRCA1/2 (CIMBA). Cancer Epidemiol. Biomarkers Prev 21(1): 134–147.

15. Rajasekaran R, Sudandiradoss C, Doss CGP, Sethumadhavan R (2007) Identification and in silico analysis of functional SNPs of the BRCA1 gene. Genomics 90(4): 447–452.

16. Krupa R, Sliwinski T, Morawiec Z, Pawlowska E, Zadrozy M et al (2009) Association between polymorphisms of the BRCA2 gene and clinical parameters in breast cancer. Exp. Oncol 31(4): 250–251.

17. National Comprehensive Cancer Network NCCN Clinical Practice Guidelines in Oncology [NCCN Guidelines®]. Genetic/Familial High-Risk Assessment: Breast and Ovarian. Available: http://www.nccn.org/professionals/physician_gls/pdf/genetics_screening.pdf.