BRIEF REPORT

A novel de novo *BRCA2* mutation of paternal origin identified in a Spanish woman with early onset bilateral breast cancer

Orland Diez · Sara Gutiérrez-Enríquez ·
Carmen Mediano · Miriam Masas ·
Cristina Saura · Neus Gadea · Judith Balmaña

Abstract Germ line mutations in either of the two major breast cancer predisposition genes, *BRCA1* and *BRCA2*, account for a significant proportion of hereditary breast/ovarian cancer. Identification of breast cancer patients carrying mutations in any of these genes is primarily based on a positive family history of breast/ovarian cancer or early onset of the disease. In the course of mutation screening of the *BRCA1* and *BRCA2* genes in a hospital based series of patients with risk factors for hereditary breast/ovarian cancer, we identified a novel germ line mutation in the *BRCA2* gene (c.51dupA) in a patient with early onset bilateral breast cancer and no family history of the disease. None of her parents carried the mutation, and paternity was confirmed. Subsequent molecular analysis demonstrated that the mutation was a novel de novo germ line mutation located in the paternal allele of the *BRCA2* gene.

Keywords BRCA2 · De novo mutation · Hereditary breast cancer

O. Diez (✉) · M. Masas
Oncogenetics Laboratory, University Hospital Vall d'Hebron,
Barcelona, Spain
e-mail: Odiez@vhebron.net

O. Diez · S. Gutiérrez-Enríquez
Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain

C. Mediano
Genetics Unit, University Hospital Vall d'Hebron,
Barcelona, Spain

C. Saura · N. Gadea · J. Balmaña
Medical Oncology Department, University Hospital Vall
d'Hebron, Barcelona, Spain

Introduction

It is currently estimated that 5–10% of all breast cancers are hereditary and a proportion of them attributable to mutations in several highly penetrant susceptibility genes. Mutations in the tumor-suppressor genes *BRCA1* (MIM#113705) and *BRCA2* (MIM#600185) confer an increased risk of developing breast cancer and ovarian cancer [1, 2].

Family history is essential for identifying individuals at risk for mutations in the *BRCA* genes in the clinics. Hallmark characteristics include a personal or family history of early onset breast cancer, multiple family members diagnosed with breast or ovarian cancers, multiple generations affected, breast cancer bilaterality, the occurrence of male breast cancer, and ethnicity (descent from a population with high incidence of founder mutations, such as Ashkenazi Jewish or Icelandic ancestry).

A negative family history, however, does not exclude the presence of a germ line mutation in *BRCA1* or *BRCA2* genes. Indeed, it may reflect a small family size, predominance of males in the pedigree, incomplete penetrance, premature death of at risk women, or inadequacy of the cancer history information [3]. In patients without a positive family history, mutation testing of these genes may be considered when early onset, multifocality, bilaterality, or other features suggestive of a hereditary component are present. Furthermore, recent studies show that tumor phenotype, such as the so-called triple-negative (absence of estrogen and progesterone receptors and HER2-neu expression) breast cancer, is an indicator that may help to identify candidates for *BRCA1* mutation testing, especially among young women without family history [4], who otherwise would not have been eligible.

Since the identification of *BRCA1* and *BRCA2*, more than 3,600 distinct genetic variants (including deleterious mutations, polymorphisms, and unclassified variants) have been reported for these genes (BIC database) [5]. Despite the high number of mutations identified and the large number of studies carried out world wide, to our knowledge there is only one published report of a de novo mutation in *BRCA1* [6] and three in *BRCA2* [7–9].

During the course of the molecular analysis of *BRCA1* and *BRCA2* on hereditary breast/ovarian cancer families and women diagnosed with breast cancer at young ages, we identified a novel de novo *BRCA2* mutation located in exon 2 in a Spanish patient with early onset bilateral breast cancer without a family history of breast/ovarian cancer. Furthermore, we ascertained the presence of the mutation in the paternal allele.

Personal and family history

The proband was a 38-year-old woman who had been diagnosed at the ages of 27 and 37 with bilateral infiltrating ductal carcinomas of the breast. The first tumor was estrogen and progesterone receptor negative, and the second one was estrogen and progesterone receptor positive, and HER2 negative.

No other breast or ovarian cancers were present in the pedigree (Fig. 1). Following genetic counseling, blood and tumor samples were collected from the proband for mutation screening.

Mutation identification

Genomic DNA was prepared from 10 ml EDTA blood samples according to standard procedures. Analysis of the entire coding and flanking *BRCA1/2* sequences were carried out by direct sequencing (Abi Prism 3130 DNA Sequencer, Applied Biosystems).
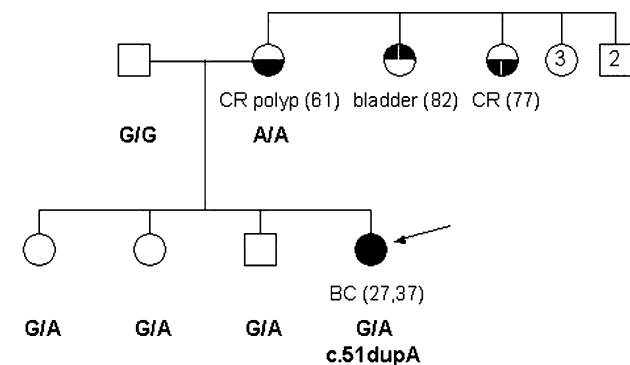
Fig. 1 Family pedigree: *BC* breast cancer; *CR* colorectal cancer. Between parenthesis: age at diagnosis. The *BRCA2* −26G/A and wt/279insA genotypes are indicated

Mutation screening of the *BRCA2* gene in the proband indicated in exon 2 the presence of 1 bp duplication (c.51dupA, according to HGVH nomenclature) (c.279_280insA according to BIC nomenclature) (*BRCA2* GenBank entry: NM_000059). The insertion results in a frame shift and a premature termination of translation at codon 30 in exon 3 of *BRCA2*. As far as we know this mutation has not been previously reported by the Breast Cancer Information Core or another database, and it has not been published before.

After identification of the mutation, the analysis was then carried out on DNA samples obtained from the proband's mother, father, and siblings. Surprisingly, neither of her parents was found to carry the mutation, suggesting that this germ line alteration had occurred de novo. The presence of the mutation in the proband was confirmed on an independent DNA sample obtained from peripheral blood leukocytes, and in DNA extracted from micro dissected archival breast cancer tissue. However, no apparent LOH was identified in the tumor.

Paternity confirmation

To confirm paternity, a set of highly polymorphic short tandem repeat (STR) markers was analyzed by QF-PCR analysis using the ANEUFAST Kit (Genomed Ltd). The markers included in the kit were D21S1414, D21S1446, D21S1411, D21S1435, D13S631, D13S305, D13S634, D13S258, D18S525, D18S391, D18S386, D18S390, AMXY, X22, HPRT, DXYS218, and SRY. PCR amplification and fragment analysis were performed according to the manufacturer's instructions. Markers were analyzed on an ABI Prism 3130 DNA Sequencer (Applied Biosystems).

All markers proved to be highly informative, consistent with the reported paternity with high probability. This result confirmed that the duplication identified in the proband was a de novo germ line mutation in *BRCA2* that occurred during parental germ-cell gametogenesis.

Parental origin of the mutation

To determine whether the mutation had occurred on the maternal or paternal allele, we analyzed a polymorphism located near the site of mutation in the 5′UTR part of the *BRCA2* gene (−26G > A according to HGVH numbering, −203G > A according to BIC). This single nucleotide polymorphism was fully informative because the father was homozygous GG, and the mother was homozygous AA (Fig. 1).

An allele specific PCR was performed using primers designed to distinguish between the two SNP alleles, and therefore the allele carrying the mutation from the wild-type allele. Specific primers were designed using the

software Primer3 in combination with MFE primer to search specificity and SNP blast alignments.

The forward primer sets were designed to distinguish the −26A and the −26G allele with the last nucleotide: 5′-TC TGTTTTGCAGACTTATTTACCAAA/G-3′, respectively. The primer reverse was 5′-GTTTCCTCTTTATCCACTA GACGTTC-3′. Primer sets were tested on the samples from both the father and the mother, used as homozygote controls. PCR was carried out using standard conditions (available upon request).

The −26G allele was present in *cis* with the mutated c.51dupA allele. In accordance, the mutation was absent in the −26A allele sequence (Fig. 2). These data indicate that the *BRCA2* c.51dupA mutation was on the father's allele and also further suggest that this mutation probably arose as a de novo event in a testicular germ cell.

Discussion

We describe the identification of the *BRCA2* c.51dupA mutation in a woman affected by bilateral early onset breast cancer at the ages of 27 and 37 and no family history of the disease. No one else in the family was identified to carry this mutation, and paternity was confirmed with the analysis of polymorphic markers. We therefore conclude that the identified novel mutation in the proband is a de novo mutation.

The presence of the alteration in the paternal allele was determined by the analysis of a polymorphism located near the mutation (−26G > A), taking advantage that the father was homozygous GG, and the mother was homozygous AA for this polymorphism. The result

suggests a de novo event in a testicular germ cell, although an occurrence in the zygote can not be discarded. This is in agreement with previous findings in de novo mutations in *BRCA1* and *BRCA2*, in which the paternal origin could be ascertained [5, 8], and also in other hereditary cancer syndromes (neurofibromatosis type 1, MEN2B and retinoblastoma) [10, 11]. It has been suggested that the origin of new mutations may be influenced by both genomic imprinting effects and the increased number of cell divisions in spermatogenesis compared with oogenesis. However, other familiar cancer syndromes do not exhibit a marked bias for a paternal origin of new mutations [12].

Although this specific mutation has not previously been described, several other presumably deleterious alterations have been reported in its vicinity in exon 2 (BIC). Examples include c.245delAA (reported three times), c.254delC (reported seven times), c.264insT, c.264delT, c.265G > T, and c.265insT (reported once each), c.279delAC (reported five times), and c.291delA (reported twice), suggesting that this area of the gene could be prone to mutations.

The observation that a previous known recurrent mutation in *BRCA* genes may appear as a de novo alteration suggest the existence of hot spots for small alterations. As an example, the *BRCA2* c.3036_3039del4 mutation may have arisen on many separate occasions in various ethnic groups, according to the results of haplotype studies, and it has also been identified as a de novo mutation [8]. The authors suggested that these occurrences could be related with tandemly repeated sequences, which favor misalignments during DNA replication.

Although all germ line mutations started as a de novo mutation in some ancestor, the proportion of de novo

Fig. 2 Electropherograms of reverse sequences corresponding to the two alleles specifically amplified from the DNA of the *BRCA2* mutation carrier. The sequence of the paternal allele shows the presence of 5′UTR-26G and c.279insA genotype. The sequence of the maternal allele shows the presence of 5′UTR-26A and the wild-type (wt) genotype
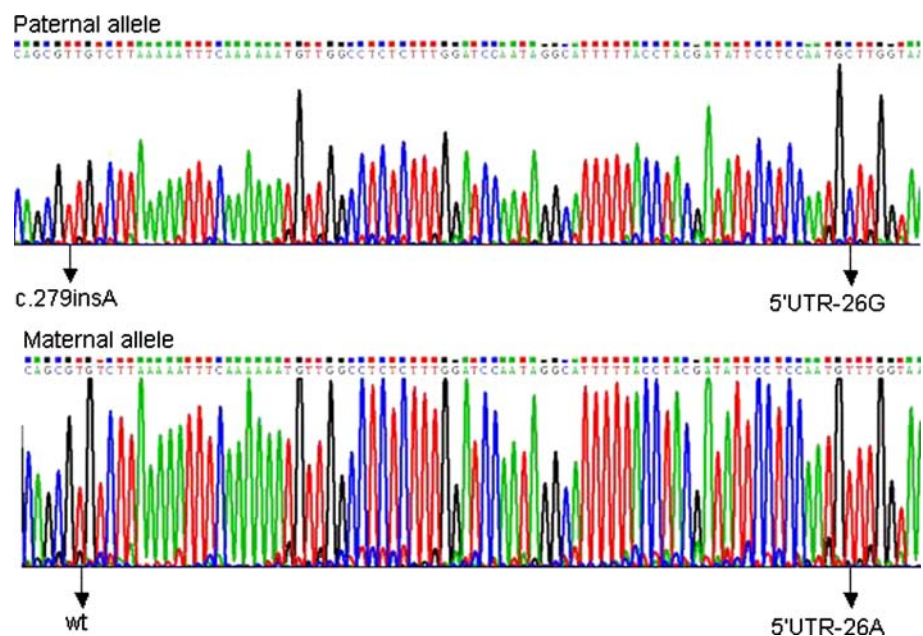
mutations among gene carriers seems to be low. To our knowledge, there is only one published report of a proven de novo mutation in the *BRCA1* gene and three in the *BRCA2* gene, and there is only one previous report of a novel de novo mutation in *BRCA2*.

Haplotype studies of founder mutations in *BRCA1* and *BRCA2* demonstrated that they remain present in the population for many generations, probably due to the fact that cancer usually occurs after the reproductive capacity has been fulfilled. However, *BRCA* mutation studies are usually performed in families with multiple cases of breast/ovarian cancer, although the de novo mutations could be particularly expected in early onset breast cancer patients with no family history of the disease. One may hypothesize whether prevalence of de novo mutations would increase in case *BRCA* genetic testing was offered to unselected patients with early onset breast cancer.

There are a number of reports of *BRCA1* and *BRCA2* germ line mutations detected in early onset breast cancer patients without family history of the disease. In a population-based series of Spanish women diagnosed with breast cancer before age 40, the prevalence of *BRCA1* and *BRCA2* germ line mutations was 11.6% [13]. Family history of breast cancer and ovarian cancer was absent or not suggestive of hereditary condition in one-fourth of mutation carriers. Lidereau et al. [14] found that 6 of 70 women (9%) with breast cancer diagnosed at age 35 or below unselected for family history carried a *BRCA1* mutation. One of them did not have any family history of breast/ovarian cancer. In a study of 254 white women from the United Kingdom, diagnosed with breast cancer before age 36, a germ line *BRCA1* or *BRCA2* mutation was identified in 15 women (6%), and only one had a family history [15]. Another recent study identified six mutations (11%) in 54 women diagnosed with high-grade, triple-negative invasive breast cancer at or before age 40, and little or no family history of breast or ovarian cancer [4]. The authors concluded that women with early onset triple-negative breast cancer are candidates for genetic testing for *BRCA1*, even in the absence of a family history of breast or ovarian cancer.

These studies underline the importance of mutation screening of the *BRCA1* and *BRCA2* genes in women with early onset breast/ovarian cancer or males with breast cancer, without a strong family history of the disease. The absence of family history might be related to non-informative kindreds (few women who lived until ages at risk of developing breast or ovarian cancer) or, albeit infrequent, the presence of a de novo mutation. Bearing in mind this possibility, the two parents of a new mutation carrier should be tested for the mutation to ascertain its origin.

This information could be useful when considering the incidence of de novo mutations of the *BRCA1* and *BRCA2* genes in genetic epidemiological studies.

Note added in proof During the publication process of the present article a de novo BRCA1 mutation has been published online: Edwards E, Yearwood C, Sillibourne J, Baralle D, Eccles D. Identification of a de novo BRCA1 mutation in a woman with early onset bilateral breast cancer. Fam Cancer. 2009 Jul 21.

References

1. Easton DF, Ford D, Bishop DT (1995) Breast and ovarian cancer incidence in *BRCA1* mutation carriers. Breast cancer linkage consortium. Am J Hum Genet 56:265–271
2. Easton DF, Steele L, Fields P, Ormiston W, Averill D, Daly PA, McManus R, Neuhausen SL, Ford D, Wooster R, Cannon-Albright LA, Stratton MR et al (1997) Cancer risks in two large breast cancer families linked to BRCA2 on chromosome 13q12–13. Am J Hum Genet 61:120–128
3. Weitzel JN, Lagos VI, Cullinane CA, Gambol PJ, Culver JO, Blazer KR, Palomares MR, Lowstuter KJ, MacDonald DJ (2007) Limited family structure and BRCA gene mutation status in single cases of breast cancer. JAMA 297:2587–2595
4. Young SR, Pilarski T, Donenberg T, Shapiro C, Hammond LS, Miller J et al. (2009) The prevalence of BRCA1 mutations among young women with triple-negative breast cancer. BMC Cancer 9:86 doi:10.1186/1471-2407-9-86
5. Breast Cancer Information Core, http://www.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic/
6. Tesoriero A, Andersen C, Southey M, Somers G, McKay M, Armes J, McCredie M, Giles G, Hopper JL, Venter D (1999) *De novo* BRCA1 mutation in a patient with breast cancer and an inherited BRCA2 mutation. Am J Hum Genet 65:567–569
7. Robson M, Scheuer L, Nafa K, Ellis N, Offit K (2002) Unique *de novo* mutation of BRCA2 in a woman with early onset breast cancer. J Med Genet 39:126–128
8. van der Luijt RB, van Zon PH, Jansen RP, van der Sijs-Bos CJ, Warlam-Rodenhuis CC, Ausems MG (2001) *De novo* recurrent germline mutation of the BRCA2 gene in a patient with early onset breast cancer. J Med Genet 38:102–105
9. Hansen TVO, Bisgaard ML, Jønson L, Albrechtsen A, Filtenborg-Barnkob B, Eiberg H, EjlertsenB NielsenFC (2008) Novel *de novo BRCA2* mutation in a patient with a family history of breast cancer. BMC Medical Genetics 9:58
10. Carlson KM, Bracamontes J, Jackson CE, Clark R, Lacroix A, Wells SA Jr, Goodfellow PJ (1994) Parent-of-origin effects in multiple endocrine neoplasia type 2B. Hum Genet 55:1076–1082
11. Kato MV, Ishizaki K, Shimizu T, Ejima Y, Tanooka H, Takayama J, Kaneko A, Toguchida J, Sasaki MS (1994) Parental origin of germ-line and somatic mutations in the retinoblastoma gene. Hum Genet 94:31–38
12. Richards FM, Payne SJ, Zbar B, Affara NA, Ferguson-Smith MA, Maher ER (1995) Molecular analysis of de novo germline mutations in the von Hippel-Lindau disease gene. Hum Mol Genet 4:2139–2143
13. de Sanjosé S, Leone M, Berez V et al (2003) Prevalence of BRCA1 and BRCA2 germline mutations in young breast cancer patients: a population-based study. Int J Cancer 106:588–593

14. Lidereau R, Eisinger F, Champeme MH, Nogues C, Bieche I, Birnbaum D, Pallud C, Jacquemier J, Sobol H (2000) Major improvement in the efficacy of BRCA1 mutation screening using morphoclinical features of breast cancer. Cancer Res 60:1206–1210

15. Peto J, Collins N, Barfoot R, Seal S, Warren W, Rahman N, Easton DF, Evans C, Deacon J, Stratton MR (1999) Prevalence of BRCA1 and BRCA2 gene mutations in patients with early-onset breast cancer. J Natl Cancer Inst 91:943–949