Biallelic Deleterious BRCA1 Mutations in a Woman with Early-Onset Ovarian Cancer


ABSTRACT

BRCA1 and BRCA2 are the most important breast and ovarian cancer susceptibility genes. Biallelic mutations in BRCA2 can lead to Fanconi anemia and predisposition to cancers, whereas biallelic BRCA1 mutations have not been confirmed, presumably because one wild-type BRCA1 allele is required during embryogenesis. This study describes an individual who was diagnosed with ovarian carcinoma at age 28 and found to have one allele with a deleterious mutation in BRCA1, c.2457delC (p.Asp821Ilefs*25), and a second allele with a variant of unknown significance in BRCA1, c.5207T>C (p.Val1736Ala). Medical records revealed short stature, microcephaly, developmental delay, and significant toxicity from chemotherapy. BRCA1 p.Val1736Ala cosegregated with cancer in multiple families, associated tumors showed loss of wild-type BRCA1, and BRCA1 p.Val1736Ala showed reduced DNA damage localization. These findings represent the first validated example of biallelic deleterious human BRCA1 mutations and have implications for the interpretation of genetic test results.

SIGNIFICANCE: Accurate assessment of genetic testing data for BRCA1 mutations is essential for clinical monitoring and treatment strategies. Here, we report the first validated example of an individual with biallelic BRCA1 mutations, early-onset ovarian cancer, and clinically significant hypersensitivity to chemotherapy. Cancer Discov; 3(4); 399–405. ©2012 AACR.

INTRODUCTION

Hereditary breast and ovarian cancer syndrome is predominantly caused by heterozygous, germline mutation in the BRCA1 or BRCA2 genes (1). Several forms of Fanconi anemia, characterized by bone marrow failure and malignancy, can be a consequence of biallelic mutations in BRCA2 (2) or biallelic mutations in genes encoding BRCA2- and BRCA1-associated proteins PALB2 and BRIP1 (3–7). Despite a frequency of approximately 1.5% in the Ashkenazi Jewish population for biallelic BRCA2 mutations, familial ovarian cancers and breast cancers may occur without a history of Fanconi anemia.

Authors’ Affiliations: 1Abramson Cancer Center, Departments of 2Medicine, 3Cancer Biology, and 4Pathology and Laboratory Medicine, 5Basser Research Center for BRCA1/2, Abramson Family Cancer Research Institute, Perelman School of Medicine, University of Pennsylvania; 6Vironika, Philadelphia, Pennsylvania; 7Lady Davis Institute and Segal Cancer Centre, Jewish General Hospital; 8Program in Cancer Genetics, Departments of Oncology and Human Genetics, and 9Research Institute, McGill University Health Centre, McGill University, Montreal, Quebec, Canada; 10Department of Medical Genetics, University of Cambridge, Cambridge; and 11Ferguson Smith Centre for Clinical Genetics, Yorkhill, Glasgow, United Kingdom; 12Cancer Epidemiology Program, H. Lee Moffitt Cancer Center, Tampa, Florida; 13Cancer Genetic Risk Assessment Program, Bryan Hemming Cancer Care Center, San Francisco, California; 14Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota; 15Huntsman Cancer Institute and Department of Dermatology, University of Utah School of Medicine, Salt Lake City, Utah

Note: Supplementary data for this article are available at Cancer Discovery Online (http://cancerdiscovery.aacrjournals.org/).

Corresponding Authors: Susan M. Domchek, Abramson Cancer Center, University of Pennsylvania, 3400 Civic Center Boulevard, Philadelphia, PA 19104. Phone: 215-615-3360; Fax: 215-615-3349. E-mail: susan.domchek@uphs.upenn.edu; and Roger A. Greenberg, Perelman School of Medicine, University of Pennsylvania, 421 Curie Boulevard, Philadelphia, PA 19104-6160. Phone: 215-746-2738; Fax: 215-573-2486; E-mail: rogergr@mail.med.upenn.edu

doi: 10.1158/2159-8290.CD-12-0421

©2012 American Association for Cancer Research.
the BRCA1 mutations, 185delAG [Human Genome Variation Society (HGVS) c.68_69delAG] and 5382insC (HGVS c.5266dupC), no homozygous or compound heterozygote carriers of these mutations have been reported. Although Brca1 nullizygosity results in embryonic lethality in mice (8), genetically engineered mice harboring biallelic mutations that correspond to human cancer-associated missense mutations within the BRCA1 carboxyl-terminal (BRCT) domain are viable through adulthood and display highly penetrant cancer susceptibility (9). These findings raise the possibility that a partially functional BRCA1 allele in trans with a deleterious truncating mutation (or with a similarly partially functioning mutation) in BRCA1 could be present within the same individual and could contribute to familial cancer susceptibility in humans. Herein, we document the presence of a functionally deleterious BRCA1 BRCT domain missense alteration in trans with a pathogenic BRCA1 alteration in a woman with dysmorphic features and early-onset ovarian carcinoma.

RESULTS

The proband (Fig. 1A, ego 28, arrow) presented at age 28 with stage IV papillary serous ovarian carcinoma. Medical records revealed a history of microcephaly, short stature (adult height of 150 cm), and developmental delay with limited speech at age 4 years. A review of pictures provided by the family showed coarse features with low anterior hairline, macrognathia, a prominent nasal bridge, and small alae nasi. She did not have obvious abnormalities of her thumbs...
Biallelic BRCA1 mutations

Table 1. Loss of heterozygosity from the index family

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Description</th>
<th>Germline BRCA1</th>
<th>Taqman result</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>S2504</td>
<td>Cell line</td>
<td>WT/WT</td>
<td>A/A</td>
<td>No LOH</td>
</tr>
<tr>
<td>S2366</td>
<td>Tumor (breast)</td>
<td>WT/WT</td>
<td>A/A</td>
<td>No LOH</td>
</tr>
<tr>
<td>Ego 1</td>
<td>Lymphoblasts</td>
<td>WT/p.Val1736Ala</td>
<td>A/G</td>
<td>No LOH</td>
</tr>
<tr>
<td>Ego 10</td>
<td>Lymphoblasts</td>
<td>WT/p.Val1736Ala</td>
<td>A/G</td>
<td>No LOH</td>
</tr>
<tr>
<td>Ego 9</td>
<td>Tumor (Ovarian)</td>
<td>WT/p.Val1736Ala</td>
<td>G/G</td>
<td>LOH at WT</td>
</tr>
<tr>
<td>Ego 28</td>
<td>Tumor (Ovarian)</td>
<td>p.2576delC/p.Val1736Ala</td>
<td>A/G</td>
<td>No LOH</td>
</tr>
</tbody>
</table>

NOTE: Ovarian/primary peritoneal cancer tumor blocks from Family A egos 1 and 9 showed that LOH had occurred at the wild-type BRCA1 allele with retention of the p.Val1736Ala allele. “A” at position 5207 represents the wild-type allele and “G” at position 5207 represents the Val1736Ala allele. Conversely, in ego 28, who carried germline BRCA1 2576delC and p.Val1736Ala alterations in trans, the ovarian tumor did not display LOH at either allele. Abbreviation: LOH, loss of heterozygosity; WT, wild type.

and had a normal complete blood count at the time of her cancer diagnosis. Neither ataxia nor telangiectasias were documented. This individual was found to have a known deleterious mutation in BRCA1 reported as 2576delC (HGVS c.2457delC; p.Asp821Ilefs*25) and a variant of unknown significance (VUS) in BRCA1 (HGVS c.5207T>C) p.Val1736Ala, as well as a VUS in BRCA2 (HGVS, c.971G>C; p.Arg324Thr).

Treatment with carboplatin [target area under the concentration versus time curve in mg/mL × min (AUC) of 5] and paclitaxel (175 mg/m²) resulted in significant toxicity requiring hospitalization due to fever and grade 4 neutropenia [absolute neutrophil count (ANC) nadir of 160/mm³], as well as grade 3 anemia (hemoglobin nadir of 7.8 g/dL) and grade 4 thrombocytopenia (nadir of 3,000/mm³), for which she received red blood cell and platelet transfusions. She also developed grade 3 nausea, diarrhea, and mucositis. As a result of the excess toxicity, carboplatin and paclitaxel were discontinued after 2 cycles. She received no further therapy and died 6 months after her diagnosis. Extreme sensitivity to the interstrand crosslinking agent carboplatin is not typically observed in biallelic BRCA1 mutation carriers (10–12), but it is seen in biallelic BRCA1-mutant cells and mice, suggesting that both BRCA1 alleles were compromised for DNA repair function.

The mother of ego 28 was diagnosed with ovarian cancer at age 53 and died at 55. A maternal great aunt (Fig. 1A, Family A, ego 1) was diagnosed with breast and ovarian cancers at ages 59 and 69, respectively, and a contralateral breast cancer at age 76. A second maternal great aunt (ego 9, sister of ego 1) was diagnosed with primary peritoneal cancer at age 53 and died at 55. A second maternal great aunt (ego 1, maternal great aunt of ego 1) was diagnosed with breast cancer at age 56 and died at 57. Notably, both carried the BRCA1 p.Val1736Ala variant VUS but not the known pathogenic mutation BRCA1 2576delC (HGVS c.2457delC). Additional genetic testing in the family revealed that the brother of the proband (ego 27) carries the BRCA1 c.2457delC mutation and the paternal lineage also had multiple cases of early-onset breast cancer. To investigate this variant further, we were able to obtain pedigrees on 11 additional families with the BRCA1 p.Val1736Ala VUS. Nine of these pedigrees that had additional genotyping of family members were used to assess cosegregation using methods described by Thompson and colleagues (ref. 13; a representative pedigree is shown in Fig. 1B, and characteristics of the families are detailed in Supplementary Table S1). The combined OR in favor of p.Val1736Ala being pathogenic was 234:1, assuming the age-specific penetrance estimated in Antoniou and colleagues (14). Loss of heterozygosity (LOH) analysis was conducted on genomic DNA extracted from BRCA1 p.Val1736Ala mutation-positive tumors using a custom designed TaqMan assay (Table 1 and Supplementary Fig. S1). Ovarian/primary peritoneal cancer tumor blocks from Family A egos 1 and 9 showed LOH at the wild-type BRCA1 allele with retention of the p.Val1736Ala allele. Conversely, in ego 28, who carried germline BRCA1 2576delC and p.Val1736Ala alterations in trans, the ovarian tumor did not display LOH at either allele, suggesting that p.Val1736Ala expression is not selected against in tumors.

The BRCA1 BRCT residue Val1736 is conserved across 18 different vertebrate species (Fig. 2A). In contrast with other BRCT residues that exhibit cancer-associated point mutations, structural models predict that Val1736 does not make direct contact with phosphopeptide ligands (Fig. 2B). Rather, Val1736 resides in a hydrophobic pocket, which may affect the stability of residues Pro1749 and Cys1697, both of which are required for BRCT function in DNA repair and tumor suppression. Transfection of a DNA double-strand break (DSB) reporter cell line (ref. 15; Supplementary Fig. S2) with an epitope-tagged carboxy-terminal region of BRCA1 revealed that a wild-type (WT) BRCT fragment was observed at more than 80% of DSBs, whereas the fragment containing p.Val1736Ala was reduced to less than 40% (P = 0.0029), intermediate to the WT protein and known BRCT-mutant p.Pro1749Arg, which was present at less than 20% of DSBs (P = 0.0017; Fig. 2C and D). Similarly, coimmunoprecipitation experiments with the same epitope-tagged BRCA1 fragments showed significantly diminished interaction between p.Val1736Ala and RAP80, a BRCA1 BRCT-interacting protein, in comparison with WT BRCT-containing fragments (Fig. 2E). Consistent with these results, overexpression of the WT BRCT fragment acted as a dominant-negative allele by reducing...
IR-induced RAD51 foci formation and homology-directed DSB repair by a significantly greater extent than overexpression of BRCA1 fragments containing either the BRCT domain mutations p.Pro1749Arg or p.Val1736Ala (Supplementary Fig. S3).

We are aware of only one previous report of biallelic deleterious mutations in BRCA1 in humans. In this report, a Scottish woman was found to be homozygous for BRCA12800delAA (HGVS c.2681_2682delAA, p.Lys894Thrfs*8; ref. 16). This individual was diagnosed with breast cancer at age 32 and subsequently developed a contralateral breast cancer. Homozygosity for this mutation was plausible particularly because it is a founder mutation in the studied population (17). Nevertheless, this report has long been questioned because potential primer bias in PCR-based genotyping could have led to preferential amplification of the putative mutant allele and hence masking of true heterozygosity (18). Because of the importance of this single report for the interpretation of our own results, we resequenced peripheral blood lymphocyte DNA from the reported biallelic carrier and found that only one BRCA1 allele harbored the designated mutation c.2681_2682delAA, whereas the other allele was found to be WT at this position (Fig. 3A).
Biallelic BRCA1 mutations

and B). Therefore, the purported homozygous carrier was in actuality heterozygous for a BRCA1 mutation.

**DISCUSSION**

Here, we report the first individual with validated biallelic mutations in BRCA1. Compelling evidence is presented that BRCA1 p.Val1736Ala is both pathogenic and can support viability through adulthood in trans to a deleterious mutation in exon 11 of BRCA1 (BRCA1 2576delC). BRCA1 p.Val1736Ala diminishes protein–protein interaction with RAP80 and localization to DSBs and imparts cancer susceptibility independent of other BRCA1 or BRCA2 alterations. LOH analysis was also consistent with pathogenicity. Loss of the WT allele occurred in both tumors that carried the p.Val1736Ala VUS in trans to WT BRCA1; however, LOH did not occur in the ovarian cancer of the proband (ego 28), which was compound heterozygous for p.Val1736Ala and 2576delC, indicative of a scenario in which selective pressure did not exist to delete either pathogenic allele.

Several features of the index patient were uncharacteristic for monoallelic BRCA1 mutation carriers. In addition to the aforementioned developmental delay, microcephaly, and short stature, ovarian cancer was diagnosed earlier than the age of 30, which is unusual for BRCA1 mutation carriers (19). The patient also had extreme sensitivity to the interstrand crosslinking agent carboplatin, a characteristic not typically displayed in BRCA1 mutation carriers. In addition to the biologic implications, the findings in this study have importance to the interpretation of genetic variants. VUSs are a common finding in genetic testing for inherited cancer syndromes and pose challenges in counseling and management (27). Cooccurrence of a VUS in trans with a known deleterious BRCA1 mutation is considered a strong indication that the VUS is not clinically important (28). Our findings suggest that the presence of a BRCA1 VUS in trans with an established deleterious BRCA1 mutation should not be considered as definitive evidence against pathogenicity. This work also highlights the importance of examining multiple distinct lines of evidence when interpreting a VUS, including clinical phenotype. This lesson is particularly pertinent in the era of massively parallel DNA sequencing, as a large number of VUSs will be identified with the use of this methodology and caution will be needed in interpreting these results for clinical use.

**METHODS**

**LOH Analysis**

DNA was extracted from either cell lines or tumors following microdissection of cancer tissue to over 70% tumor (Supplementary Fig. S3). LOH was assessed by the University of Pennsylvania Genomics Facility using a custom-designed TaqMan assay to distinguish a single nucleotide alteration at nucleotide position 5207, codon 1736 from the WT allele (Table 1 and Supplementary Fig. S1).

**BRCA1 c.2681_2682delAA Resequencing**

Lymphocyte DNA from the patient was amplified by PCR using the primers F1: 5’-AACCACAGTCGGGAAACAAG-3’ and R2:
Immunofluorescence

Immunofluorescence was conducted in the DSB reporter cells as described previously (15). No additional authentication on cell lines was conducted. All analyses were carried out on unmodified images that were captured with a QImaging RETIGA-SRV camera connected to a Nikon Eclipse 80i microscope.

Disclosure of Potential Conflicts of Interest

D.E. Goldgar has an ownership interest (including patents) and royalties from BRCA1/2 gene patents. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions

Conception and design: S.M. Domchek, K.L. Nathanson, W. Foulkes, R.A. Greenberg
Development of methodology: S.M. Domchek, R.A. Greenberg
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): S.M. Domchek, J. Tang, J. Stopfer, D.R. Lill, N. Hamel, M. Tischkowitz, A. Yonker, F.J. Couch, H.R. Davidson, K.L. Nathanson, W. Foulkes, R.A. Greenberg
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): S.M. Domchek, J. Stopfer, K.L. Nathanson, R.A. Greenberg
Study supervision: S.M. Domchek, R.A. Greenberg

Acknowledgments

The authors thank R. Pilarski, A. Guy Malloy, T. Vu, S. Diaz, K. Berry, J. Homer, S. Mecas-Faxon, J. Blount, and L. Levitch for providing additional pedigrees for analysis and K. Addya for assistance with BRCA1 genotyping. The authors also thank the members of the Breast Cancer Information Core steering committee and Myriad Genetics for critical discussion.

Grant Support

This study was supported by funding from the Basler Research Center for BRCA1/2 (to R.A. Greenberg, K.L. Nathanson, and S.M. Domchek). R.A. Greenberg was also supported by funding from 1R01CA138835-01 from the NCI, an American Society for Research Scholar Grant, DOD Award BC111503P1, a pilot grant from the joint FCCC-UPENN Ovarian specialized program of research excellence (SPORE), and funds from the Abramson Family Cancer Research Institute. K.L. Nathanson is supported by the Breast Cancer Research Foundation (BCRF) and the Rooney Family Foundation. S.M. Domchek is supported by Susan G. Komen for the Cure. W.D. Foulkes receives funding from Susan G. Komen for the Cure and the Weekend to End Breast Cancer (Jewish General Hospital). This work was also supported in part by NIH grant CA161667 and an NCI SPORE in breast cancer to the Mayo Clinic (P50-CA116201).

Received September 18, 2012; revised December 16, 2012; accepted December 21, 2012; published OnlineFirst December 26, 2012.

REFERENCES


Biallelic Deleterious \textit{BRCA1} Mutations in a Woman with Early-Onset Ovarian Cancer

Susan M. Domchek, Jiangbo Tang, Jill Stopfer, et al.