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BRIEF REPORT

Collective evidence supports neutrality of BRCA1 V1687I, a novel sequence variant in the conserved THV motif of the first BRCT repeat

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Abstract Unambiguous classification of *BRCA1* and *BRCA2* variants of uncertain significance (VUS) is a challenging task that vexes health care providers and has profound implications for patients and their family members. Numerous VUS have been described to date, which await assessment of their functional, hence clinical, impact. As a result of a routine *BRCA1/BRCA2* mutational screening, we identified a previously unreported *BRCA1* sequence alteration [c.5178G>A (V1687I)] in a patient diagnosed with early onset triple negative breast cancer. The sequence alteration falls in the invariant THV motif of the BRCT domain. To investigate its significance, we applied an integrated approach that, in addition to genetic and histopathological data, included in silico analyses, comparative structural modeling and verification of BRCT-mediated interactions. In line with web-based algorithms that predicted the benign nature of BRCA1 V1687I, the three-dimensional model of the BRCA1 V1687I BRCT domain did not reveal any major structural changes relative to its wild-type counterpart, thus suggesting that BRCA1 V1687I has a negligible impact on both the local architecture and the overall stability of the protein. Consistently, the BRCA1 V1687I protein was properly expressed and localized to the nucleus, and it was still capable of binding three BRCT-interacting, DNA damage response, and repair partner proteins, namely BRIP1/FANCJ, CtIP, and Abraxas. Our collected evidence suggests that, although occurring in a highly conserved region, the BRCA1 V1687I variant is likely a benign sequence alteration.

Keywords BRCA1 · BRCT domain · Variants of uncertain significance · Triple negative breast cancer

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Introduction

Identification of sequence variants with unknown effect on protein function is one possible outcome of *BRCA1/BRCA2* genetic testing (with rates varying based on patients' ethnicity) [1]. These so-called variants of uncertain significance (VUS) comprise missense changes, small in-frame deletions and insertions, and alterations in non-coding intervening sequences or in regulatory regions [2]. Accurate assessment of their disease relevance is critical. Indeed, individuals carrying a sequence alteration of proven pathogenicity can benefit from ad hoc surveillance, prophylactic and/or therapeutic options while those who carry biologically (and clinically) irrelevant variants can be relieved from unnecessary distress [2].

At present, a multifactorial likelihood method [3], which integrates evidences derived from multiple independent sources, is considered as the most reliable quantitative approach to determine whether a variant is disease associated or not. The model has been repeatedly implemented to incorporate additional independent features in the final computation [4]. Although genetic evidence remains the gold standard for variant classification, the inherent rarity of individual VUS and consequent scarcity of epidemiological data often limit the statistical power of the analysis thus impeding an accurate estimate of disease causality. Ongoing efforts are aimed at widening the range of information evaluated in the model by including data derived from in silico and in vitro analyses [2, 5].

Due to the pleiotropic biological roles of the BRCA1 and BRCA2 proteins, currently available in vitro assays can measure only a few functions and/or are relevant to variants that localize to specific domains [2]. For BRCA1, most clinically relevant missense mutations reside in the functionally and structurally well-characterized N-terminal RING finger domain and C-terminal tandem BRCT repeats (BRCT domain) [6–8]. The RING finger is involved in the heterodimerization with the cognate domain of the partner protein BARD1 [9] to form an active E3 ubiquitin ligase [10]. The BRCT domain is a phosphoprotein-binding module that mediates interaction with several BRCA1 functional partners, such as BRIP1/FANCJ [11], CtIP [12], and Abraxas [13], and plays a critical role in DNA damage response and repair processes [14].

We have previously reported on the application of an integrated approach to assess the structural and functional repercussions of BRCA1 V1688del, a founder sequence alteration described in families of Italian descent [15, 16]. The BRCA1 V1688del in-frame deletion occurred in a region of the BRCT that is key to the stability of the domain [7] and our analysis demonstrated its detrimental effect on protein localization and function [16].

Here, we apply an analogous comprehensive strategy to establish the effect of a novel sequence variant, BRCA1 V1687I, which occurs immediately upstream and was identified in one patient with early onset triple negative breast cancer. Overall, we present circumstantial evidence that supports the neutral significance of BRCA1 V1687I.

Materials and methods

Patient and family history

Between 1995 and 2011, 858 breast and/or ovarian cancer patients were tested for *BRCA1/2* mutations at the Familial Breast and Ovarian Cancer Center in Modena, Italy. Of them, 186 (22 %) were found to carry clinically ascertained mutations. The *BRCA1* c.5178G>A (V1687I) variant was identified in the family MO678, originating from Northern Italy and selected for *BRCA1/2* mutational screening based on previously described criteria [17]. Written informed consent to collect a blood specimen for a study on breast cancer susceptibility was obtained from the proband.

BRCA1 and *BRCA2* gene analysis

Mutation screening was carried out by direct sequencing. In brief, genomic DNA was extracted from peripheral blood lymphocytes of the index individual using the Invisorb Universal Kit (Invitek, Berlin, Germany). *BRCA1* and *BRCA2* genes were PCR amplified with primers spanning all coding sequences and intronic–exonic junctions. Amplification products were subjected to capillary sequencing using an ABI 3100 sequence analyzer (Applied Biosystems, Foster City, CA, USA).

Screen for large genomic rearrangements in the *BRCA1* gene was done by multiplex ligation-dependent probe amplification (MLPA) using SALSA MLPA kits P002B and P087 (MRC-Holland, Amsterdam, the Netherlands) according to the manufacturer's instructions. Amplified, carboxyfluorescein (FAM)-labeled products were separated on ABI 3100 genetic analyzer. Data analysis was performed by Peak Scanner Software v1.0 (Applied Biosystems, Foster City, CA, USA). Duplication was suspected when the ratio between sample and control peak height was >1.30; deletion was suspected when the ratio between sample and control peak height was <0.70. Positive MLPA results were confirmed by long-range PCR using the Expand Long Template PCR System (Roche Diagnostics Corporation, Indianapolis, IN, USA).

Histopathology and immunohistochemistry

Five micrometer sections obtained from the formalin-fixed paraffin-embedded breast tumor block were stained with

hematoxylin and eosin. Receptor status, the presence of HER-2/neu amplification, and proliferation index were evaluated by immunohistochemistry according to the avidin–biotin method using a Ventana Benchmark autostainer. The antibodies used were: anti-estrogen receptor (ER), clone 6F11, and anti-progesterone receptor (PR), clone 1E2, (Ventana Medical System, Tucson, AZ, USA); anti-HER2/neu, clone CB11 (Novocastra, Newcastle upon Tyne, UK); and anti-Ki 67, clone mib-1 (Dako, Carpinteria, CA, USA).

ER and PR receptor status was evaluated based upon the percentage (%) of nuclear immunoreactivity with respect to the total number of tumor cell nuclei, independently from the staining intensity. Nuclear staining $\geq$10 % was scored as a positive result. HER2/neu staining results were scored as follows: samples with no membrane staining (0), partial membrane staining in >10 % of tumor cells (1+), weak to moderate complete membrane staining in >10 % of tumor cells (2+), strong complete membrane staining in >10 % of tumor cells (3+).

Prediction programs

To predict the impact of BRCA1 V1687I on protein function, the following programs were used: Align-Grantham Variation Grantham Deviation (A-GVGD) (http://agvgd.iarc.fr/), Panther (http://www.pantherdb.org), Polymorphism Phenotyping (PolyPhen) v2 (http://genetics.bwh.harvard.edu/pph2/), and Sorting Intolerant from Tolerant (SIFT) (http://sift.bii.a-star.edu.sg/).

Structural modeling

Comparative structural modeling was done using the MODELLER software package [18]. The crystallographic structure of the BRCA1 wild-type (wt) BRCT domain (PDB code: 1jnx) was used as a template to create the homology model of the BRCA1 V1687I BRCT domain [7]. The final model was validated using the Verify3D Structure Evaluation Server (http://nihserver.mbi.ucla.edu/Verify_3D), as well as by Ramachandran plot analysis.

cDNA constructs, cell culture, and transfection

The single nucleotide substitution at the relevant site was generated using the Quick Change site-directed Mutagenesis kit (Stratagene, La Jolla, CA, USA) and pcDNA3.1-Myc/3xHA-wtBRCA1 [16] as a template.

293T (HEK 293T/17), HeLa, and U2OS cells, obtained from the American Type Culture Collection (ATCC) (Manassas, VA, USA), were maintained in Dulbecco's modified Eagle Medium (DMEM) supplemented with 10 % fetal bovine serum (Gemini Bio-Products, West Sacramento, CA, USA) and 1 % penicillin/streptomycin (Life Technologies, Carlsbad, CA, USA), at 37 °C in a humidified incubator containing 10 % (vol/vol) CO_2. Upon growth to 50 % confluence, cells were transfected with the relevant plasmids (and pEGFP-C2 plasmid [19], when specified) using Fugene 6 transfection reagent (Roche Diagnostics Corporation, Indianapolis, IN, USA) and harvested after 40–48 h.

Gamma irradiation

Forty hours after transfection, cells were either mock treated or exposed to ionizing radiation [IR (9 Gy)] using a Gammacell 40 Exactor (Nordion, Ottawa, ON, Canada), and were allowed to recover for 4 h before being harvested.

Immunoprecipitation (IP) and western blot analysis

IP and western blot analysis were carried out as previously described [16]. The following rabbit polyclonal antibodies were used: anti-Abraxas (generous gift from S. Elledge), anti-BRIP1 I82 [19], anti-HA (ab9110) (Abcam, Cambridge, MA, USA). The following mouse monoclonal antibodies were used: anti-CtIP 14-1 [20] (generous gift from R. Baer); anti-GFP, clone C163 (Life Technologies, Carlsbad, CA, USA); anti-HA.11, clone 16B12 (Covance, Emeryville, CA, USA); anti-Myc, clone 4A6 (Upstate Biotechnology Inc., Lake Placid, NY, USA); anti-alpha tubulin, clone DM1A (Sigma-Aldrich, St. Louis, MO, USA). Immunodetection was carried out using HRP-conjugated sheep anti-mouse IgG, donkey anti-rabbit IgG, or protein A (GE Healthcare, Piscataway, NJ, USA).

Results

During a routine screening for *BRCA1* and *BRCA2* mutations, we identified a sequence change in *BRCA1* exon 17 (c.5178G>A) that causes a valine to isoleucine (V–I) substitution at position 1,687 and that was not described in the Breast Cancer Information Core (BIC) database (http://research.nhgri.nih.gov/bic/) (Fig. 1a). The alteration, found in a 49-year-old woman diagnosed with breast cancer at age 33, was unique among all breast/ovarian cancer families screened and absent in 50 unaffected control individuals. No co-occurrence of the variant with any known clinically relevant *BRCA1* mutations was observed. Family history revealed the absence of other breast cancer cases and showed only one additional malignancy (stomach cancer) in the proband's father (Fig. 1b). Blood samples from other family members were not available for analysis. Pathological evaluation of the tumor specimen revealed a

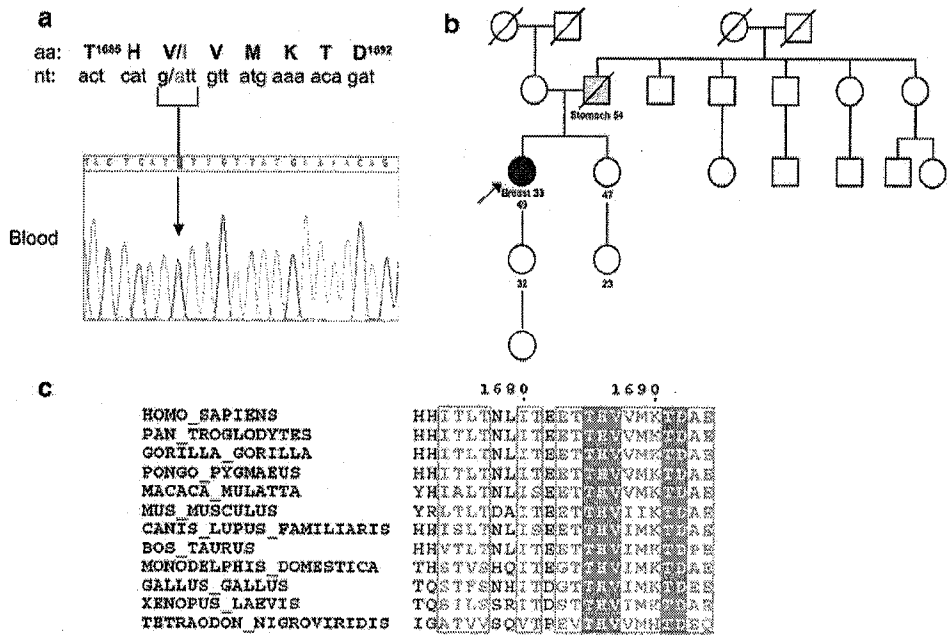

Fig. 1 V1687I is a novel BRCA1 variant occurring in a highly conserved region of the BRCT domain. **a** Electropherogram showing the nucleotide change (G>A) at position 5,178. The nucleotide/amino acid correspondence is detailed in the *upper panel*. **b** Pedigree of the family MO678. The proband, carrier of the BRCA1 V1687I sequence variant, is marked by an *arrow*. **c** Sequence alignment of a region of the first BRCT repeat in 12 vertebrates. Evolutionary fully conserved amino acids are shadowed in *red*. The alignment of multiple orthologous full-length BRCA1 protein sequences was done using ClustalW [24] and formatted using ESPript [25]. Reference sequences for cDNA and protein numbering are GenBank U14680.1 and GenPept NP_009225, respectively. The name of the sequence variant is according to BIC nomenclature guidelines

highly proliferative (ki67 55 %) medullary breast carcinoma that, as typical of BRCA1-mutated tumors, lacked expression of ER and PR, as well as HER2/neu amplification.

As genetic data did not suffice, we set out to garner complementary evidence that could help us establish the significance of the BRCA1 V1687I variant. Alignment of multiple orthologous BRCA1 protein sequences showed that valine 1687 (V1687) is an extremely conserved residue that lies in the THV motif of the first BRCT repeat (Fig. 1c). Nevertheless, all the computational algorithms (Align-GVGD, Panther, PolyPhen-2, and SIFT) we employed to estimate the potential impact of the variant on the function of the corresponding protein predicted that BRCA1 V1687I is likely benign.

To inquire the discrepancy between the strong phylogenetic conservation of V1687 and the web-based predictions, we used the available X-ray crystal structure of the BRCA1 wt BRCT domain [7] as a template and constructed a theoretical model of the BRCA1 V1687I BRCT that could help us assess any possible structural variations caused by the amino acid substitution. The model did not reveal any major structural changes relative to the wt crystal structure (Fig. 2). As isoleucine is only one carbon atom bigger than valine and both amino acids are

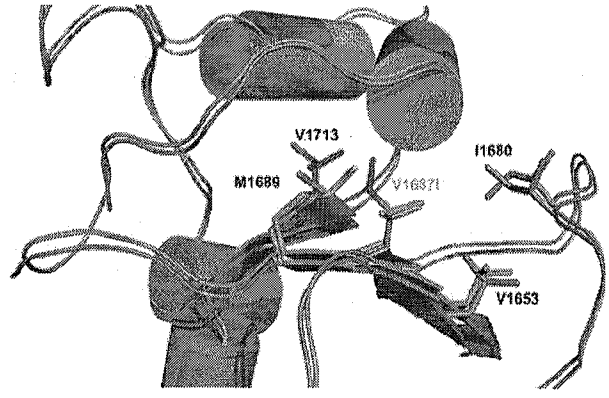

Fig. 2 Structural model of the BRCA1 V1687I BRCT. Superposition of the BRCA1 V1687I BRCT domain homology model, shown in *brown*, and the BRCA1 wt BRCT domain crystal structure (PDB code: 1jnx), depicted in *green*. The side chains of the hydrophobic residues contacting the mutated amino acid and forming the stabilizing hydrophobic region around it are shown. The model suggests that, due to both the similar hydrophobic nature and the comparable size of the two residues, little, if any, structural variations are necessary to replace the valine side chain with the isoleucine side chain. The figure was created using PyMOL (http://www.pymol.org)

hydrophobic in nature, the substitution did not appear to cause any major rearrangement within the hydrophobic core that characterizes the BRCT repeat region around

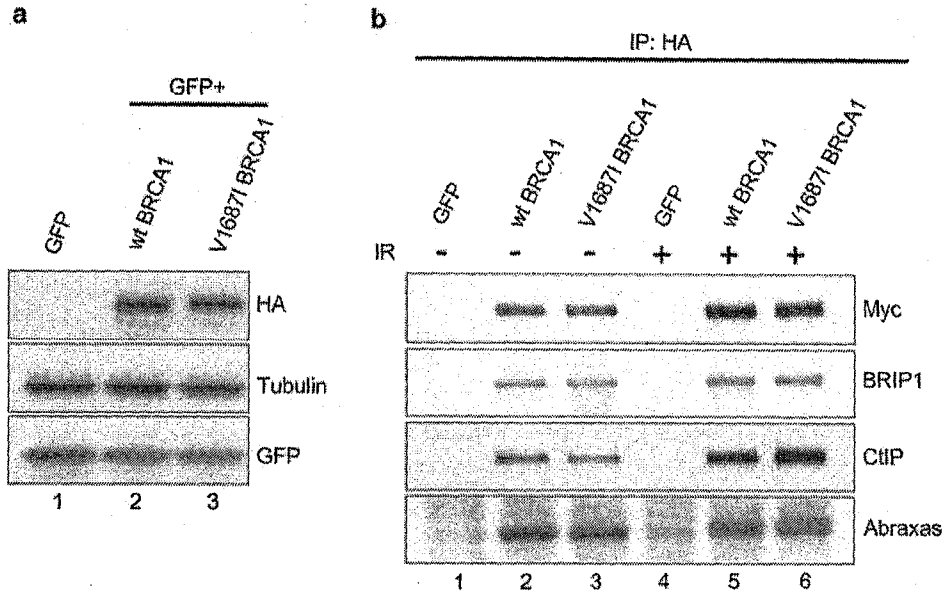

Fig. 3 BRCA1 V1687I retains interactions with known BRCT-binding partner proteins. 293T cells were transfected with a GFP-encoding plasmid alone or in combination with a construct encoding an N-terminally Myc/3HA-tagged BRCA1 wt or BRCA1 V1687I protein. **a** Cell lysates were resolved by SDS-PAGE and probed with the indicated antibodies. The expression of the BRCA1 V1687I protein appears fairly comparable to that of the wt counterpart. **b** Cell lysates from mock- (*lanes 1–3*) or IR-treated (*lanes 4–6*) cells were subjected to anti-HA IP followed by western blot analysis with the indicated antibodies. Note the similar composition of the BRCA1 wt- and BRCA1 V1687I-associated protein complexes

V1687. Conceivably, BRCA1 V1687I leaves the local architecture and overall stability of the protein largely unchanged.

Because pathogenic mutations that reside in the BRCT domain are known to impair BRCT-mediated associations and functions [7], we asked whether the V1687I variant had any effect on BRCT-mediated binding to known partners. For this purpose, we generated a construct encoding a Myc and HA epitope-tagged BRCA1 protein bearing the relevant amino acid substitution and carried out transient transfection experiments comparing the expression and properties of the BRCA1 V1687I protein versus those of the wt counterpart in different cell lines.

In our settings, the BRCA1 V1687I protein was easily detected (Fig. 3a) and it properly localized to nuclear foci (data not shown). In addition, IP experiments with anti-tag antibodies in 293T cells revealed that both, exogenously expressed, BRCA1 wt and BRCA1 V1687I proteins bound BRIP1/FANCJ, CtIP, and Abraxas (Fig. 3b, lanes 2, 3). Analogous results were obtained in HeLa and U2OS cells and were confirmed in reciprocal IP experiments using anti-BRIP1 and anti-CtIP antibodies (data not shown). We did not detect any significant difference in the composition of BRCA1 wt- and BRCA1 V1687I-associated protein complexes when cells were subjected to IR-induced DNA damage (Fig. 3b, lanes 5, 6). Thus, BRCA1 V1687I does not seem to affect the integrity of the BRCT domain as a phosphopeptide-binding module.

Discussion

We report on the identification and analysis of a novel BRCA1 sequence alteration, V1687I (c.5178G>A) that falls in the conserved THV motif of the first BRCT repeat of BRCA1. V1687 lies in the third strand of a four-stranded β sheet and it packs against a hydrophobic patch formed by several residues on two adjacent α-helices (Fig. 2) and known to have a stabilizing effect on the protein [7]. Reportedly, V1687 is also a central component of an extended, stabilizing hydrogen-bonding network spanning across a large area of the BRCT domain [21].

The occurrence of the V1687I variant in a highly conserved region of a key functional domain of the BRCA1 protein, together with the patient's early age at diagnosis and the tumor histology and receptor status compatible with a BRCA1-deficient phenotype, prompted us to further investigate the potential repercussions of BRCA1 V1687I on protein structure and function via a multimodal approach. Notably, pathogenicity has been suggested for two neighboring variants, BRCA1 V1688del [15] and BRCA1 H1686Q [22], which are both associated with functional impairment ([16] and our unpublished observations).

The results of our integrated approach revealed a complete agreement among in silico predictions, comparative structural modeling, and analysis of BRCT-mediated binding, and suggested that the valine to isoleucine substitution does not affect the structure and function of the

encoded protein. Particularly, homology modeling of the BRCA1 V1687I BRCT clarified the computational predictions by illustrating how the conserved V1687 residue can be mutated to isoleucine without causing any disruption in either the hydrophobic environment surrounding the side chain of the residue or the extended hydrogen-bonding network that the residue is part of. Moreover, the congruent finding that the BRCA1 V1687I protein is still capable of interacting, as efficiently as the wt protein, with three major BRCA1 partners in DNA damage response and repair, holds with its plausible proficiency in processes known to be crucial to BRCA1 tumor suppression. Altogether our collected evidence bolsters the view that BRCA1 V1687I is a benign sequence alteration.

Assessment of the cancer risk associated to *BRCA1* and *BRCA2* VUS is an overwhelmingly important clinical problem that requires a multidisciplinary approach. The usefulness of indirect evidence contributed by sources of data other than genetic (e.g., in silico and in vitro analyses) to achieving VUS classification is a well-accepted notion. Recently, substantial progress has been made [5] to meet the requirement of rigorous validation, which is mandatory to warrant integration of functional assay results into VUS classification models. In this context, low-throughput analyses like ours, focused on a single or a few variants and aimed at elucidating yet unclear structure–function relationships of BRCA1, might provide valuable qualitative evidence to complement scanty genetic information and, hence, contribute to VUS categorization.

In light of the data presented herein, and consistent with the younger age of onset reported for sporadic triple negative breast cancer compared to the other subtypes [23], the breast cancer of the proband of family MO678 is conceivably sporadic. Alternatively, it could be accounted for by a pathogenic mutation in other breast cancer predisposing gene(s), which we were unable to test.

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Conflict of interest The authors declare that they have no conflict of interest.

References

1. Domchek S, Weber BL (2008) Genetic variants of uncertain significance: flies in the ointment. J Clin Oncol 26(1):16–17. doi:10.1200/JCO.2007.14.4154
2. Spurdle AB (2010) Clinical relevance of rare germline sequence variants in cancer genes: evolution and application of classification models. Curr Opin Genet Dev 20(3):315–323. doi:10.1016/j.gde.2010.03.009
3. Goldgar DE, Easton DF, Deffenbaugh AM, Monteiro AN, Tavtigian SV, Couch FJ (2004) Integrated evaluation of DNA sequence variants of unknown clinical significance: application to BRCA1 and BRCA2. Am J Hum Genet 75(4):535–544. doi:10.1086/424388S0002-9297(07)62706-2
4. Chenevix-Trench G, Healey S, Lakhani S, Waring P, Cummings M, Brinkworth R, Deffenbaugh AM, Burbidge LA, Pruss D, Judkins T, Scholl T, Bekessy A, Marsh A, Lovelock P, Wong M, Tesoriero A, Renard H, Southey M, Hopper JL, Yannoukakos K, Brown M, Easton D, Tavtigian SV, Goldgar D, Spurdle AB (2006) Genetic and histopathologic evaluation of BRCA1 and BRCA2 DNA sequence variants of unknown clinical significance. Cancer Res 66(4):2019–2027. doi:10.1158/0008-5472.CAN-05-3546
5. Iversen ES Jr, Couch FJ, Goldgar DE, Tavtigian SV, Monteiro AN (2011) A computational method to classify variants of uncertain significance using functional assay data with application to BRCA1. Cancer Epidemiol Biomarkers Prev 20(6):1078–1088. doi:10.1158/1055-9965.EPI-10-1214
6. Brzovic PS, Meza JE, King MC, Klevit RE (2001) BRCA1 RING domain cancer-predisposing mutations Structural consequences and effects on protein–protein interactions. J Biol Chem 276(44):41399–41406. doi:10.1074/jbc.M106551200M106551200
7. Williams RS, Green R, Glover JN (2001) Crystal structure of the BRCT repeat region from the breast cancer-associated protein BRCA1. Nat Struct Biol 8(10):838–842. doi:10.1038/nsb1001-838nsb1001-838
8. Williams RS, Chasman DI, Hau DD, Hui B, Lau AY, Glover JN (2003) Detection of protein folding defects caused by BRCA1-BRCT truncation and missense mutations. J Biol Chem 278(52):53007–53016. doi:10.1074/jbc.M310182200M310182200
9. Wu LC, Wang ZW, Tsan JT, Spillman MA, Phung A, Xu XL, Yang MC, Hwang LY, Bowcock AM, Baer R (1996) Identification of a RING protein that can interact in vivo with the BRCA1 gene product. Nat Genet 14(4):430–440
10. Hashizume R, Fukuda M, Maeda I, Nishikawa H, Oyake D, Yabuki Y, Ogata H, Ohta T (2001) The RING heterodimer BRCA1-BARD1 is a ubiquitin ligase inactivated by a breast cancer-derived mutation. J Biol Chem 276(18):14537–14540
11. Cantor S, Drapkin R, Zhang F, Lin Y, Han J, Pamidi S, Livingston DM (2004) The BRCA1-associated protein BACH1 is a DNA helicase targeted by clinically relevant inactivating mutations. Proc Natl Acad Sci USA 101(8):2357–2362
12. Yu X, Wu LC, Bowcock AM, Aronheim A, Baer R (1998) The C-terminal (BRCT) domains of BRCA1 interact in vivo with CtIP, a protein implicated in the CtBP pathway of transcriptional repression. J Biol Chem 273(39):25388–25392
13. Wang B, Matsuoka S, Ballif BA, Zhang D, Smogorzewska A, Gygi SP, Elledge SJ (2007) Abraxas and RAP80 form a BRCA1 protein complex required for the DNA damage response. Science 316(5828):1194–1198
14. Manke IA, Lowery DM, Nguyen A, Yaffe MB (2003) BRCT repeats as phosphopeptide-binding modules involved in protein targeting. Science 302(5645):636–639
15. Malacrida S, Agata S, Callegaro M, Casella C, Barana D, Scaini MC, Manoukian S, Oliani C, Radice P, Barile M, Menin C, D'Andrea E, Montagna M (2008) BRCA1 p.Val1688del is a deleterious mutation that recurs in breast and ovarian cancer families from Northeast Italy. J Clin Oncol 26(1):26–31. doi:10.1200/JCO.2007.13.2118
16. De Nicolo A, Parisini E, Zhong Q, Dalla Palma M, Stoeckert KA, Domchek SM, Nathanson KL, Caligo MA, Vidal M, Cusick ME,

Garber JE (2009) Multimodal assessment of protein functional deficiency supports pathogenicity of BRCA1 pV1688del. Cancer Res 69(17):7030–7037. doi:10.1158/0008-5472.CAN-09-1440
17. Cortesi L, Turchetti D, Marchi I, Fracca A, Canossi B, Rachele B, Silvia R, Rita PA, Pietro T, Massimo F (2006) Breast cancer screening in women at increased risk according to different family histories: an update of the Modena Study Group experience. BMC Cancer 6:210. doi:10.1186/1471-2407-6-210
18. Sali A, Blundell TL (1993) Comparative protein modelling by satisfaction of spatial restraints. J Mol Biol 234(3):779–815. doi:10.1006/jmbi.1993.1626
19. De Nicolo A, Tancredi M, Lombardi G, Flemma CC, Barbuti S, Di Cristofano C, Sobhian B, Bevilacqua G, Drapkin R, Caligo MA (2008) A novel breast cancer-associated BRIP1 (FANCJ/BACH1) germ-line mutation impairs protein stability and function. Clin Cancer Res 14(14):4672–4680. doi:10.1158/1078-0432.CCR-08-0087
20. Yu X, Baer R (2000) Nuclear localization and cell cycle-specific expression of CtIP, a protein that associates with the BRCA1 tumor suppressor. J Biol Chem 275(24):18541–18549
21. Karchin R, Monteiro AN, Tavtigian SV, Carvalho MA, Sali A (2007) Functional impact of missense variants in BRCA1 predicted by supervised learning. PLoS Comput Biol 3(2):e26. doi:10.1371/journal.pcbi.0030026
22. Giannini G, Capalbo C, Ottini L, Buffone A, De Marchis L, Margaria E, Vitolo D, Ricevuto E, Rinaldi C, Zani M, Ferraro S, Marchetti P, Cortesi E, Frati L, Screpanti I, Gulino A (2008) Clinical classification of BRCA1 DNA missense variants: H1686Q is a novel pathogenic mutation occurring in the ontogenetically invariant THV motif of the N-terminal BRCT domain. J Clin Oncol 26(25):4212–4214. doi:10.1200/JCO.2008.18.2089 (author reply 4214–4215)
23. Bauer KR, Brown M, Cress RD, Parise CA, Caggiano V (2007) Descriptive analysis of estrogen receptor (ER)-negative, progesterone receptor (PR)-negative, and HER2-negative invasive breast cancer, the so-called triple-negative phenotype: a population-based study from the California cancer Registry. Cancer 109(9):1721–1728. doi:10.1002/cncr.22618
24. Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG (2007) Clustal W and Clustal X version 2.0. Bioinformatics 23(21):2947–2948. doi:10.1093/bioinformatics/btm404
25. Gouet P, Courcelle E, Stuart DI, Metoz F (1999) ESPript: analysis of multiple sequence alignments in PostScript. Bioinformatics 15(4):305–308