Received: 9 April 2018 | Revised: 31 August 2018 | Accepted: 11 September 2018
DOI: 10.1002/mc.22910

BRIEF COMMUNICATION

WILEY Molecular Carcinogenesis

Unraveling the molecular effect of a rare missense mutation in *BRIP1* associated with inherited breast cancer

Carolina Velázquez[1] | Eva M. Esteban-Cardeñosa[1] | Enrique Lastra[2] | Luis E. Abella[3] | Virginia de la Cruz[3] | Carmen D. Lobatón[1] | Mercedes Durán[1] | Mar Infante[1]

[1] Cancer Genetics Group, Institute of Genetics and Molecular Biology (UVa-CSIC), Valladolid, Spain

[2] Unit of Genetic Counseling in Cancer, Complejo Hospitalario de Burgos, Burgos, Spain

[3] Unit of Genetic Counseling in Cancer, Hospital Universitario Rio Hortega, Valladolid, Spain

Correspondence
María Del Mar Infante, Grupo de Genética del Cáncer, Instituto de Biología y Genética Molecular (CSIC-UVa), Sanz y Forés 3. 47003 Valladolid, Spain
Email: minfante@ibgm.uva.es

Funding information
Regional Government of Castilla and León co-financed by European Social Fund (ESF), Grant number: (ORDEN EDU/1083/2013); University of Valladolid

BRIP1 is a component of the Fanconi Anemia/BRCA pathway responsible for DNA reparation via helicase activity. Some heterozygous variants in *BRIP1* could contribute to Hereditary Breast Cancer through a defective DNA repair. The clinical utility of *BRIP1* mutations in a familial cancer context is compromised by the conflicting interpretation of "variants of uncertain significance" (VUS). Defining the clinical significance of variants identified in genetic tests is a major challenge; therefore, studies that evaluate the biological effect of these variants are definitely necessary. To contribute to this purpose, we have characterized the variant c.550G>T of *BRIP1*, a missense mutation with little evidence about its pathogenicity. Since Human Splicing Finder™ predicts the creation of a new exonic splicing enhancer site we decided to perform cDNA analysis revealing that the c.550G>T mutation located in exon 6 led to an aberrant transcript causing exon 5 skipping. Our results demonstrate that the c.550G>T *BRIP1* variant disrupts normal splicing, causing exon 5 skipping. Considering that the exon 5 encodes the helicase domain of BRIP1, it is expected an alteration of the function. This finding enhances the interpretation of this VUS, suggesting a potential pathogenic effect.

KEYWORDS
aberrant splicing, *BRIP1*, germline mutations, hereditary breast and ovarian cancer

1 | INTRODUCTION

Explaining the genetic predisposition to Hereditary Breast Cancer is a doubly complex task: it is not only necessary to find mutations, but also to define their pathogenesis. Risk assessment, early detection, and treatment strategies are the key principles of genetic counseling in Hereditary Breast Cancer cases.

BRIP1 is a DNA helicase and a tumor suppressor gene with an important role in Homologous Recombination by direct binding with BRCA1/2 proteins.[1,2]

Genetic testing for *BRIP1* germline mutations in breast cancer cases with a strong family history can contribute to clinical management[3] through early detection, prevention measures, and therapeutic selection. This clinical utility is controversial in the case of "variants of unknown significance," (VUS).

Splicing alteration is usually assigned to flanking exon-intron boundary variants, but single nucleotide substitutions in exonic regions can also affect the mRNA processing.[4] The mechanism could be based on creating novel splicing enhancers sites, or activating cryptic splicing sites.[5] Since the management of VUS in moderate-penetrance genes

Present Address for Eva M. Esteban-Cardeñosa: Fundación Pública Galega de Medicina Xenómica-SERGAS, Santiago de Compostela, Spain.

like *BRIP1* is a challenge, evaluating their potential splicing alteration has to be encouraged.

2 | MATERIALS AND METHODS

2.1 | Case report

A Hereditary Breast Cancer family with a VUS in *BRIP1* was recruited by the Genetic Counseling Unit of Castile & Leon, Spain. Briefly, three relatives were diagnosed with breast cancer: a woman with breast cancer at 52 (III.2); a woman with breast cancer at the age of 52 (III.3); and a woman with bilateral breast cancer at 46 and 52 (II.4) (Figure 1).

The *BRIP1* mutation c.550G>T (p.Asp184Tyr) was firstly identified in III.3 case in a next-generation sequencing panel containing more than 4800 clinically relevant genes (TruSight™ One Sequencing Panel, Illumina, San Diego, CA). Nor pathogenic neither likely pathogenic variants in other susceptibility genes have been detected. Due to its pathogenicity remains unclear, the RNA of index case (IV.2) and other available were used to further characterize the variant.

The frequency of the variant was evaluated in a control population—320 healthy blood donors from the National DNA Bank without familial cancer history. All participants provided written, informed consent. Ethical committee approval was obtained. DNA samples were extracted using MagNAPure Compact Nucleic Acid Isolation Kit.

2.2 | Detection of point mutations

The probands II.1 and IV.2 were recruited through Genetic Counseling and specifically screened for the c.550G>T mutation by Sanger sequencing.

To discard any other cause of splicing alteration, exonic and flanking intronic regions of exons 4, 5, 6, and 7 of *BRIP1* were sequenced with the BigDye Terminator Sequencing Kit v3.1 (Applied Biosystems, Foster City, CA) on an ABI 3100 DNA sequencer (Applied Biosystems). Furthermore, the entire intron 5 was sequenced in order to discard any other distant splicing variant. The 320-control group was screened for c.550G>T mutation by High Resolution Melting Analysis in LightCycler480 (Roche, Basilea, Suiza). Mutation nomenclature was based on the NM_032043.2 GenBank reference sequence.

2.3 | Detection of large genomic rearrangements (LGRs)

Multiplex ligation-dependent probe amplification (MLPA) analysis with SALSA P240-A2 BRIP1/CHEK1 Kit (MRC-Holland, Amsterdam, the Netherlands) was used to discard any germline LGR in the index case. The PCR products were separated by electrophoresis on an ABI3130 and analyzed by the MRC Coffalyser software (MRC-Holland).

2.4 | RNA isolation and RT-PCR

RNA was extracted from peripheral blood lymphocytes using the GeneMATRIX Human Blood RNA Purification Kit (EURx, Gdánsk, Poland). The cDNA synthesis was performed using the Transcriptor First Strand cDNA Synthesis Kit (Roche), according to the manufacturer's instructions.

We designed the primer pair −4 Forward (5′ CAGATGAGGGCG-TAAGTGA-3′) and 7 Reverse (5′- CGTCCTCCGGAGCTCTCTAG-3′)—to evaluate the transcript spanning exons 4-7 of *BRIP1* using the two available cDNA of the c.550G>T carriers and other three control cDNAs from non-carriers. The RT-PCR reaction consisted of a 1X Buffer A, 0.5 µM forward and reverse primers, 0.32 mM dNTP Mix, 1 Unit of Kappa Taq DNA Polymerase, 12 µL of the cDNA generated in a final volume of 100 µL .The cycling conditions were denaturation at 95°C for 3 min, 35 cycles at 95°C for 30 s, 56°C for 30 s, and 72°C for 45 s, followed by a final extension at 72°C for 10 min.

Products were separated in low melting 2% agarose gel and visualized with Red Safe™ staining. Isolated bands were extracted using NucleoSpin® Gel and PCR Clean-up (Macherey-Nagel, Düren, Germany) and subsequently sequenced.

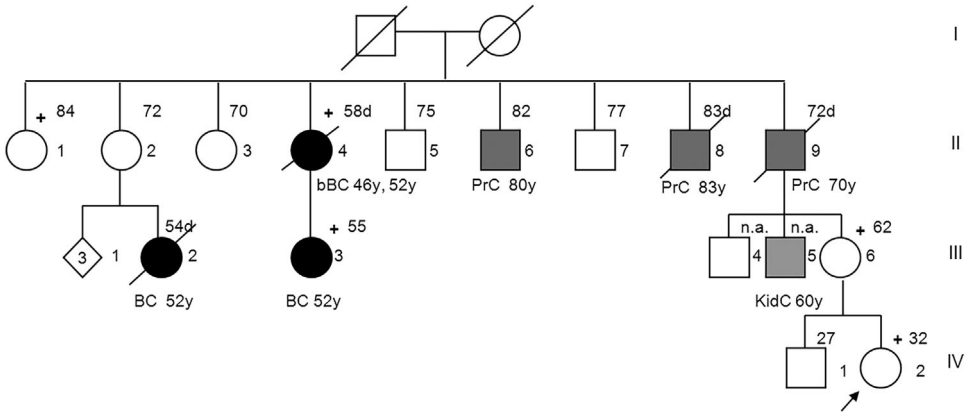

FIGURE 1 Patient pedigree. Affected individuals are indicated with filled symbols, whereas unaffected relatives are indicated by open symbols. " − ": wild type allele; " + ":*BRIP1* c.550G>T (p.Asp184Tyr) mutation. Type of cancer and age at diagnosis are indicated when known. Black symbol is BC (breast cancer) or bBC (bilateral breast cancer). Dark grey symbol is PrC (Prostate cancer), and light grey symbol is KidC (Kidney cancer). A slashed circle/square indicates a deceased individual. Actual ages or age of dead "d" are indicated behind each individual, or n.a. if it is not available

2.5 | Bioinformatics analysis

The mutation was analyzed for potential pathogenic effects using the following in silico tools: PolyPhen (http://genetics.bwh.harvard.edu/pph2/), SIFT (http://sift.jcvi.org/), Human Splicing Finder™ 3.0 (HSF) (http://www.umd.be/HSF3/index.html), and ESE finder 3.0 (http://krainer01.cshl.edu/cgi-bin/tools/ESE3/esefinder).

3 | RESULTS

3.1 | Mutation identification

A missense mutation c.550G>T (p.Asp184Tyr) in the *BRIP1* suppressor gene was identified in a Breast Cancer family (Figure 1). This variant was also identified in Breast Cancer and Lynch Syndrome families.[6–8]

The allele frequency of c.550G>T *BRIP1* variant in ExAC database is 0.00016. To further define the frequency of the mutation we screened 320 controls. None of them carried the mutation, confirming that c.550G>T *BRIP1* is a rare missense variant. The calculated Odds ratio, 6.0282, would indicate a link between the variant and the disease. This data may be taken carefully since the confidence interval is very wide (0.2442-148.8215) and there is no statistical significance ($P = 0.27$) possibly due to the very low frequencies and the small sample sizes.

3.2 | Variant In silico analysis

Concerning BRIP1 protein, c.550G>T variant replaces aspartic acid with tyrosine at codon 184 (p.Asp184Tyr). In silico analyses suggest that the amino acid change is likely to be harmful at a structural and functional protein level: SIFT "Deleterious," PolyPhen-2 "Probably Damaging." In addition to the predictions related to the amino acid change, we also evaluated its potential effect on splicing. In this respect, HSF predicts a new donor splicing enhancer site. Specifically, ESE finder indicates that the missense variant breaks an Exonic Splicing Enhancer site (ESE) used by SRSF2, thus the recruitment of the splicing machinery could be affected.

3.3 | RT-PCR analysis

To confirm HSF and ESE predictions BRIP1 transcript including exons 4, 5, 6, and 7 was amplified by RT-PCR. Interestingly, mutation carriers transcripts revealed two bands: a 585 basepairs (bp) band corresponding with the expected wildtype fragment and an aberrant transcript band (464 bp), while controls only showed the wild-type band. The RT-PCR products from one of the carriers and one of the controls can be compared in Figure 2A. The presence of an aberrant transcript band endorses the splicing alteration predicted but the most striking result was the exon 5 skipping revealed by sequencing (Figure 2A). To guarantee the splicing event, we performed the RT-PCR using the RNA of the other carrier evidencing the same pattern (Figure 2B). To confirm exon 5 skipping, we designed a forward primer overlapping exons 4 and 6. Hypothetically, the primer only matched an aberrant transcript without exon 5, where the transition from the sequence of exon 4 and 6 took place. We carried out a PCR using the primer that sits in between exon 4 and 6 (4/6 Fw: 5′ CAACTTGTCAAGATTA-GAAAACG 3′) and the 7 reverse primer with the RT-PCR products as template. Only the carrier sample showed the 303 bp PCR product (Figure 2B).

3.4 | LGRs and other point mutations

Considering that an exon 5 germline deletion in heterozygosis could explain the observed result, we carried out a MLPA. No large genomic rearrangements were detected (Supplemental figure S1).

In addition, neither the entire 5-6 intron nor intronic flanking regions of exons 4, 5, 6, and 7 showed variants that could be responsible for exon 5 skipping.

3.5 | Segregation studies

We investigated if the c.550G>T *BRIP1* variant segregated with Breast Cancer phenotype in the family (Figure 1). Among the three affected women, only two were available; both of them carried the mutation. On the other hand, another three women carried the mutation but, as far as we know, none of them had developed cancer. The family study suggests an incomplete segregation, typical of low-penetrance cancer genes, which could be conferring a significant risk in this pedigree with several breast and prostate cancer cases.[9]

4 | DISCUSSION

The usefulness of cancer genetics tests is compromised by the detection of VUS. Clinical management is difficult when the effect of a VUS remains unclear; therefore, molecular characterization studies are highly needed.

A VUS in *BRIP1*, c.550G>T (p.Asp184Tyr), was detected in a Breast Cancer family. This is a rare missense variant according with the low frequency in population databases (rs201047375, ExAC 0. 016%) and the absence in our control group (320 samples). It has been previously reported in other cancer studies but neither segregation analysis nor functional assays has been performed.

Considering any mutation could potentially modify the splicing process,[4,10] we examined the variant with HSF and ESE finder tools. As a result, an alteration of an ESE was predicted, a possible underlying cause of anomalous exon skipping. In fact, SR proteins are well-characterized RNA binding proteins that promote exon inclusion by binding to ESEs.[11] According with this hypothesis, we performed a transcript analysis revealing an aberrant band. Intriguingly, the sequencing of this band confirmed an exon 5 skipping. This event could be consistent with the inactivation of the ESEs placed on the adjacent exon.[12] It is suggested that the SRSF2 protein would be regulating exon 5 inclusion through its interaction with the ESEs placed on exon 6. Consequently, when the ESE is disrupted, SRSF2 fails to recruit splice machinery and the exon 5 skipping happens.

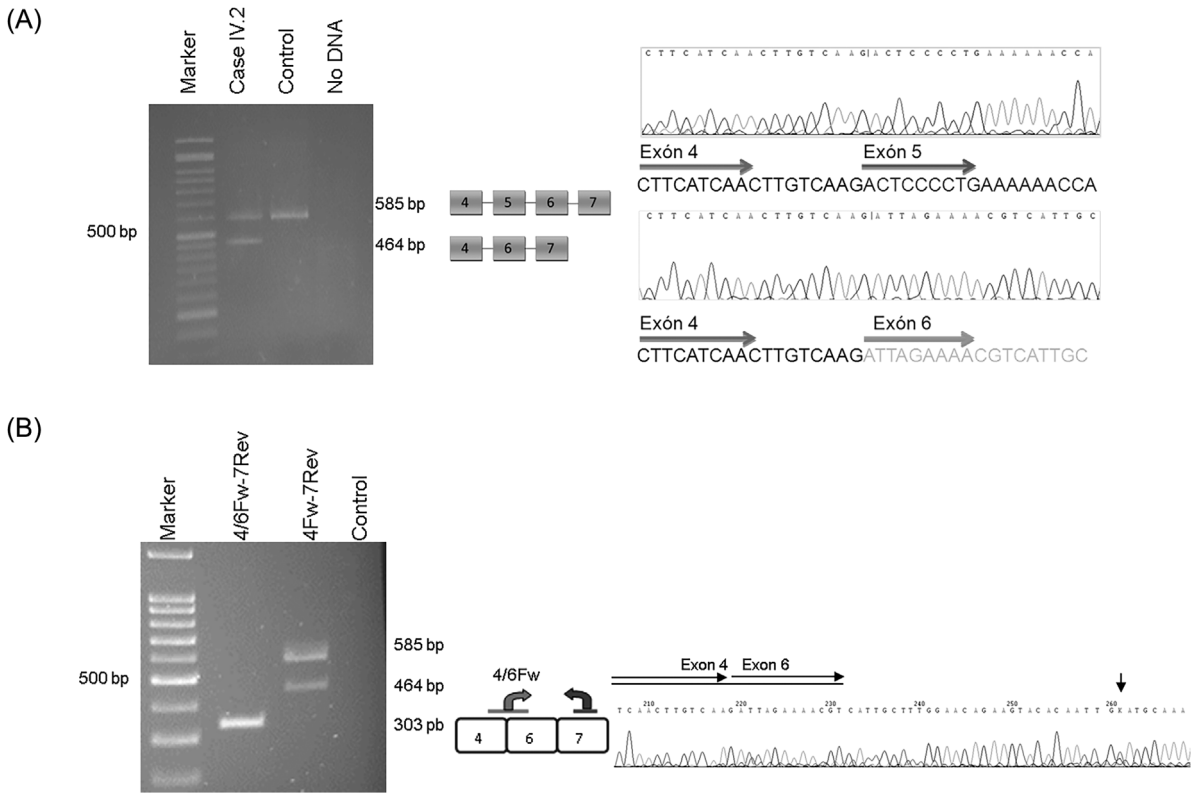

FIGURE 2 RT-PCR analysis of *BRIP1* c.550G>T (A) Agarose gel electrophoresis of PCR spanning exons 4-7 revealed two bands: upper band (1) the expected wild-type allele of 585 bp and lower band (2) of 464 bp corresponding to exon 5 deletion. Sequence of the two alternative transcripts (1 and 2) revealing an in-frame deletion of exon 5 of *BRIP1* gene. B, Amplification of aberrant transcript using primers 4/6Fw-7Rev in the index case (IV.2) and in a control, using the RT-PCR product with primer pair 4Fw-7Rev. The chromatogram shows the transition from exon 4 to exon 6. The arrow shows the position of nucleotide 550

To reinforce the assumption that c.550G>T *BRIP1* variant is responsible for the discovered splicing disruption, it seems essential to exclude any other genetic cause. For this purpose, firstly we had corroborated no genomic exon 5 deletion using MLPA; secondly, we had further checked the entire 5-6 intron sequence and intronic flanking sequence of exons 4, 5, 6, and 7, ruling out any other concerning splice variant.

These results support the hypothesis that the VUS changes the sequence of an ESE element in exon 6, disrupting the splicing. This statement would be consistent with the exon 5 skipping observed in both mutation carriers.

At protein level, exon 5 encodes part of the Helicase ATP-binding domain,[13] suggesting a probably impairment of protein function which could affect the DNA repair efficiency and, consequently, modulate Breast Cancer risk. To further argue the relationship between the c.550G>T *BRIP1* mutation and Breast Cancer predisposition, RNA binding assays and functional studies will be required.

As a consequence of BRIP1 disruption, it may be inferred a sensitivity of the carriers to chemotherapeutic agents like PARP inhibitors.[14] In this particular case, c.550G>T *BRIP1* mutation provokes an exon 5 skipping; this exon loss would compromise the protein functionality, leading to defective Homologous Recombination Repair. Given this premise, c.550G>T *BRIP1* mutation could be considered as a candidate PARP inhibitor response biomarker, improving the therapy selection.

Based on pedigree information, an incomplete segregation of the mutation with Breast Cancer is observed, consistent with the condition of a low-penetrance gene. This incomplete penetrance suggests that modifier genes, epigenetic events and environmental factors may determine the cancer phenotype.[15]

Interestingly, the presence of three prostate cancer cases in this family would be in agreement with other studies describing the association of *BRIP1* mutations with prostate cancer cases in the context of Hereditary Breast Cancer families[16,17] One of the prostate cancers in our family (II.9) is an obligate carrier, but it could not be ascertained in the other two cases (II.6, II.8), so it would be advisable to test male relatives and follow-up of carriers.

As far as we know, this is the first attempt to prove the in silico prediction of c.550G>T *BRIP1* mutation. The analysis at RNA level confirms an exon 5 skipping, probably as a result of the creation of a new enhancer donor site of splicing. It is worth noting that an exonic variant located in exon 6 has an impact on exon 5, highlighting the complexity of the spliceosome machinery.

5 | CONCLUSIONS

In this report, we describe a Hereditary Breast Cancer family with a VUS in *BRIP1*. The cDNA study indicates that the variant completely

abolishes normal splicing by creating a novel 5′ splice enhancer site, which led to a novel transcript without exon 5.

We have contributed to the molecular characterization of the c.550G>T *BRIP1* mutation, classified as a VUS. Interestingly, the results support its pathogenicity due to the alteration of splicing rather than the amino acid substitution emphasizing how complex it is to define the contribution of missense variants to cancer predisposition and endorsing the studies at RNA level.

ACKNOWLEDGMENTS

This work was supported by the University of Valladolid, Valladolid (Spain) and The Regional Government of Castilla y León. We would also like to thank Lara Hernández and Noemí Martínez for their excellent technical support and Alan Hynds for his critical reading of this manuscript.

CONFLICTS OF INTEREST

All the authors disclose no conflicts of interest.

ORCID

Mar Infante http://orcid.org/0000-0002-7197-5249

REFERENCES

1. Brosh RM Jr., Cantor SB. Molecular and cellular functions of the FANCJ DNA helicase defective in cancer and in Fanconi anemia. Front. *Genet*. 2014;5:372.
2. Bridge WL, Vandenberg CJ, Franklin RJ, y Hiom K. The BRIP1 helicase functions independently of BRCA1 in the Fanconi anemia pathway for DNA crosslink repair. *Nat Genet*. 2005;37:953–957.
3. Venkateshwari A, Clark DW, Nallari P, et al. BRIP1/FANCJ mutation analysis in a family with history of male and female Breast cancer in India. *J Breast Cancer*. 2017;20:104–107.
4. Morrison A, Chekaluk Y, Bacares R, Ladanyi M, y Zhang L. BAP1 missense mutation c. 2054 A>T (p. E685V) completely disrupts normal splicing through creation of a novel 5′ splice site in a human mesothelioma cell line. *PLoS ONE*. 2015;10:e0119224.
5. Cartegni L, Chew SL, y Krainer AR. Listening to silence and understanding nonsense: exonic mutations that affect splicing. *Nat Rev Genet*. 2002;3:nrg775.
6. Rummel SK, Lovejoy L, Shriver CD, y Ellsworth RE. Contribution of germline mutations in cancer predisposition genes to tumor etiology in young women diagnosed with invasive breast cancer. *Breast Cancer Res Treat*. 2017;164:593–601.
7. Shirts BH, Casadei S, Jacobson AL, et al. Improving performance of multigene panels for genomic analysis of cancer predisposition. *Genet Med*. 2016;18:974–981.
8. Yurgelun MB, Allen B, Kaldate RR, et al. Identification of a variety of mutations in cancer predisposition genes in patients with suspected lynch syndrome. *Gastroenterology*. 2015;149:604–613.
9. Byrnes GB, Southey MC, y Hopper JL. Are the so-called low penetrance breast cancer genes, ATM, BRIP1, PALB2 and CHEK2, high risk for women with strong family histories? *Breast Cancer Res*. 2008;10:208.
10. Park E, Pan Z, Zhang Z, Lin L, y Xing Y. The expanding landscape of alternative splicing variation in human populations. *Am J Hum Genet*. 2018;102:11–26.
11. Pandit S, Zhou Y, Shiue L, et al. Genome-wide analysis reveals SR protein cooperation and competition in regulated splicing. *Mol Cell* 2013;50:223–235.
12. Jang HN, Lee M, Loh TJ, et al. Exon 9 skipping of apoptotic caspase-2 pre-mRNA is promoted by SRSF3 through interaction with exon 8. *Biochim Biophys Acta BBA – Gene Regul Mech*. 2014;1839:25–32.
13. Cantor SB, y Guillemette S. Hereditary breast cancer and the BRCA1-associated FANCJ/BACH1/BRIP1. *Future Oncol Lond Engl* 2011;7:253–261.
14. Lim D, y Ngeow J. Evaluation of the methods to identify patients who may benefit from PARP inhibitor use Endocr. *Relat Cancer* 2016;23:R267–R285.
15. Cooper DN, Krawczak M, Polychronakos C, Tyler-Smith C, y Kehrer-Sawatzki H. Where genotype is not predictive of phenotype: towards an understanding of the molecular basis of reduced penetrance in human inherited disease. *Hum Genet*. 2013;132:1077–1130.
16. Pilié PG, Johnson AM, Hanson KL, et al. Germline genetic variants in men with prostate cancer and one or more additional cancers. *Cancer* 2017;123:3925–3932.
17. Kote-Jarai Z, Jugurnauth S, Mulholland S, et al. A recurrent truncating germline mutation in the BRIP1/FANCJ gene and susceptibility to prostate cancer. *Br J Cancer* 2009;100:426.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Velázquez C, Esteban-Cardeñosa EM, Lastra E, et al. Unraveling the molecular effect of a rare missense mutation in *BRIP1* associated with inherited breast cancer. *Molecular Carcinogenesis*. 2019;58:156–160. https://doi.org/10.1002/mc.22910