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SHORT COMMUNICATION

New insights into the performance of multigene panel testing: Two novel nonsense variants in *BRIP1* and *TP53* in a young woman with breast cancer

Verónica Castillo-Guardiola[a,*], M Desamparados Sarabia-Meseguer[a,*], Miguel Marín-Vera[b], Ana Isabel Sánchez-Bermúdez[a], José Luis Alonso-Romero[b], José Antonio Noguera-Velasco[a], Francisco Ruiz-Espejo[a]

[a] *Genetic Diagnostic Laboratory, Department of Clinical Analyses, Clinical University Hospital Virgen Arrixaca, Ctra Murcia-Cartagena s/n. El Palmar, 30120 Murcia, Spain;* [b] *Department of Medical Oncology, Clinical University Hospital Virgen Arrixaca, Ctra Murcia-Cartagena s/n. El Palmar, 301520 Murcia, Spain*

Abstract

Li-Fraumeni syndrome is an autosomal-dominant disorder caused by germline mutations in the tumour suppressor gene *TP53.* Here we report the case of a family whose index case was a woman diagnosed with bilateral breast cancer at the age of 18 and who had a non-informative result after *BRCA1* and *BRCA2* testing. After extending the study through multigene panel testing, two clinically relevant variants in the *TP53* and *BRIP1* genes, respectively, were found. Afterwards, the patient developed a glioblastoma. Both tumours were consistent with Li-Fraumeni syndrome. Thanks to the possibility of studying different genes related with hereditary breast and ovarian cancer, it was possible to find out the gene variant that caused the early onset cancers in the patient. Furthermore, genetic counselling was provided to the index case and her family.

Keywords Li-Fraumeni syndrome, Hereditary breast and ovarian cancer (HBOC), Next generation sequencing (NGS), Novel mutations, Multigene panel testing.

Introduction

Breast cancer is the most common malignancy in women around the world. It is estimated that 5-10% of all breast cancer cases in women are linked to hereditary susceptibility due to mutations in autosomal dominant genes [1]. In fact, breast cancer is also a component of several other well-described cancer syndromes, including Li-Fraumeni syndrome (LFS), Cowden syndrome (PTEN hemartroma tumour syndrome), Peutz-Jeghers syndrome, and hereditary diffuse gastric cancer [2,3]. The most common of these syndromes is hereditary breast and ovarian cancer (HBOC) syndrome. Historically, the risk of hereditary breast and ovarian cancer (HBOC) has been linked to pathogenic variants (PVs) in *BRCA1* and *BRCA2*. However, it is now estimated that more than half of individuals with a PV who meet the National Comprehensive Cancer Network (NCCN) guidelines testing criteria for HBOC carry PVs in genes other than *BRCA1* or *BRCA2* [2]. They include high-penetrance genes like *TP53* and *PTEN* in addition to moderate and low-penetrance genes like *CHEK2, ATM, BRIP1* and *PALB2,* among others. These genes are also associated with susceptibility to other types of cancer like ovarian, pancreatic or colorectal cancer. Many of these genes are essential for the genomic stability of the cells and are functionally related to homologous recombination in DNA repair [2,4].

The emergence of next generation sequencing (NGS) has allowed the development of multigene panel testing. Consequently, clinical practice in the field of hereditary breast and ovarian cancer (HBOC) has changed since many genes are being tested in the same analysis and several variants are being found. Some of them are novel variants, so clinical

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* Corresponding author. Tel.: +34 968369441; fax: +34 968369418.
E-mail addresses: veronicacgu.88@gmail.com, sarabia@um.es

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laboratory professionals have the responsibility of classifying their pathogenicity and collaborating with doctors to decide clinical recommendations for those patients who are diagnosed with this syndrome and their relatives. Here we present two novel nonsense mutations in the *TP53* and *BRIP1* genes in a woman who was diagnosed with bilateral breast cancer (bBC) and a malignant brain tumour. A complete genetic study together with an accurate classification of these variants was essential for the diagnosis and clinical decision-making in the patient.

Materials and methods

Clinical case

We reported the case of a family whose index case (IC) was a woman from Morocco who was diagnosed with stage IV bilateral breast cancer (bBC) at the age of 18 after consulting the doctor about a palpable mass in the left breast. She belonged to a family with four sisters and two brothers and had no family history of cancer.

The mammography and ultrasound showed multiple highly suspicious malignancy microcalcifications in the left breast. The guided core needle breast biopsy and the magnetic resonance imaging (MRI) test were compatible with carcinoma. As a result, the breast cancer committee suggested a bilateral mastectomy with reconstruction. Both biopsy pieces showed bilateral infiltrating ductal carcinoma. The results of the immunohistochemistry analysis for expression levels were negative for estrogen receptor (ER) and progesterone receptor (PR) and positive for human epidermal growth factor receptor (HER2). The first-line therapy selected was docetaxel, pertuzumab and trastuzumab. After three cycles of chemotherapy she showed a full response to treatment. She completed 22 cycles and later rejected receiving any more treatments after that. Consequently, she kept going to hospital only for check-ups and computer tomography/proton emission tomography (CT/PET) scans. The CT/PET scans were normal two consecutive times separated in five months. Two months after, the patient attended the emergency department for weakness in the upper and lower left limb. Imaging tests showed three right frontal lesions. The neurosurgeons performed the complete excision of the biggest lesion. Brain biopsy showed a glioblastoma multiforme with p53 null mutations in the immunochemical analysis, which was compatible with a mutation in *TP53*. The chosen therapy was temozolamide and radiotherapy.

Methods

As soon as the patient developed a bBC, due to the early age of diagnosis (before age 35), the patient met the criteria for HBOC [5], so genetic testing of *BRCA1* and *BRCA2* was performed. The mutational screening of *BRCA1* and *BRCA2* was performed using the BRCA MASTER^{TM}Dx kit from Multiplicom and subsequent sequencing was carried out using the Illumina Miseq Platform, whose limit of detection is 0.4-4% for germline variations depending on the depth of coverage. The result was non-informative. However, due to the high index of suspicion of hereditary syndrome related to breast cancer, the genetic counselling committee decided to extend the study to include multigene panel testing through NGS [5], following agreed criteria established by that committee (breast cancer before age 25). At the time of the study, the available technology in the genome diagnosis laboratory was the BRCA Hereditary Cancer Master^{TM} Plus kit from Multiplicom and subsequent sequencing was performed using the Illumina Miseq Platform, which included the analysis of the following high and moderate penetrance genes related to HBOC: *BRCA1, BRCA2, TP53, STK11, PTEN, CDH1, ATM, MUTYH, CHEK2, PALB2, BRIP1, RAD51C, RAD51D, RAD50, BLM, NBN, EPCAM, MLH1, MSH6, PMS2, MSH2, BARD, MRE11A, MEN1, XRCC2* and *FAM175A*. To ease the bioinformatics analysis we used the Sophia Genetics Platform.

In order to determine whether the genetic variants found were previously described and to know their pathogenic significance, the following locus specific databases (LSDBs) were consulted: Human Gene Mutation Database [6], Leiden Open Variation Database [7] and ClinVar at National Center of Biotechnology information [8]. The potential clinical effect of the novel variants found was evaluated using the prediction analysis web tool Mutation Taster [9] and Mutalyzer [10].

To classify the novel variants we followed the criteria of the American College of Medical Genetics and Genomics (ACMG) [11].

The clinically relevant variants were found and confirmed by standard Sanger sequencing using BigDye terminator sequencing kits (Applied BioSystems).

Results

Two clinically relevant variants were found (Table 1). The first of these was in the *BRIP1* gene. It is located in chromosome 17 and encodes the BRCA1-interacting protein 1, a DNA helicase that interacts with the *BRCA1* BRCTs *in vivo*. It co-localizes with *BRCA1* at sites of DNA damage, and contributes to DNA repair function. The variant found, c.886G>T is in the seventh exon of the gene and is a frameshift mutation which creates a stop codon in aminoacid 296 of the protein, which means the loss of 76% of the protein, including a great part of the helicase ATP-binding domain, the complete region of interaction with *BRCA1* and three of four ion-sulfur binding domains, required for the helicase activity.

The second variant is a duplication in the fourth exon of *TP53*, a gene which codes for a transcription factor associated with cell proliferation and apoptosis: p53. The variant c.334_364dup is a frameshift mutation that creates a truncated protein with the loss of the following functional domains: the DNA-binding domain, the oligomerization domain and the carboxyl terminal domain.

None of these variants had been described in the bibliography or databases before. Notwithstanding, according to the criteria of the American College of Medical Genetics and Genomics (ACMG) we classified them as likely pathogenic and pathogenic, respectively [11]. The *BRIP1* variant met one very strong criterion as it is a nonsense variant with a strong deleterious effect, and one moderate criteria since it is located in a well-established functional domain (helicase ATP-binding). With regard to the variant in *TP53*, it met one very strong criterion due to its deleterious effect and one strong criterion since we considered it as a de novo variant, as the family history