

K3326* mutation in breast cancer

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ABSTRACT

After more than 25 years since its identification in 1995, the mutational faults in the BRCA2 gene is recognized and accepted as a high-penetrance predisposition gene for breast cancer. Besides a large number of pathogenic mutations, there are thousands of VUS (variants of uncertain significance) described, with many of them localized on C-terminus. K3326* is a rare truncating variant on the C-terminus of BRCA2, which is reported in the literature, including ClinVar and Varsome database, as a neutral polymorphism. However, there is some evidence that suggests an elevated risk of breast cancer associated with K3326* variant.

We present the case of a K3326* mutation carrier, affected patient aged 42 diagnosed with co-existing breast and ovarian cancer, suggesting that the variant is not neutral for breast and ovarian cancer risk. K3326 may need to be included in SNP panels and further investigated for breast cancer risk estimation.

Keywords: breast cancer, ovarian cancer, BRCA2, VUS, mutation, genetic testing, c.9976A>T, K3326*, PARP inhibitors

INTRODUCTION

BRCA2 gene, acknowledged as the breast cancer predisposition gene, presents a huge number of VUS (variants of uncertain significance) – more than 4600 variants, with more than 1800 mutations on C-terminus domain of the BRCA2 protein. One of the mutations on the C-terminus domain is the c.9976A>T (p.Lys3326Ter, rs11571833) located on exon 27 – the final exon of the BRCA2 gene. The mutation consists in the transposition of the A to T, which determines a nonsense mutation (K3326*) and disparition of the last 93 amino acid of the BRCA2 protein.

The study of K3326 mutation is significant in the carrier patient counseling and management due to its implications as a possible pathogenic mutation (although it is currently listed as a benign mutation) and secondly for the recommendation of the adequate treatment.

The main role BRCA2 plays in human genome

stability makes it an important component in hereditary cancers. Despite decades of work, many variants are still catalogued as “unknown clinical significance”, being a challenge for clinicians who provide genetic counseling to the patients identified as carriers of these variants. Actually, most patients who undergo testing for a familial cancer pattern will have one or more variants of unknown significance (VUS), resulting in assessment difficulties and indefinite guidance for patient management, one of these VUS being the K3326* mutation

BRCA2 loss of function variants play a fundamental part in deciding patient prognosis and management patterns. Tumours lacking BRCA proteins exhibit enhanced susceptibility to PARP-inhibitor therapy combined with platinum salts. The inherited mutations concerning the BRCA2 protein throughout the human population is vital to be studied in order to increase the capacity of clinicians to foresee and treat cancers connected to BRCA2 mutation.

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ASSESSMENT

BRCA2 is an essential mediator of DNA double-strand break repair in animals. There are two possible pathways for double-strand breaks to be repaired: nonhomologous end joining (NHEJ) and homologous recombination (HR or HDR). BRCA2 is an essential component in the HDR repair pathway.

The BRCA2 gene consists of 27 exons which contain 70 kb of genomic DNA, detrmng a 3418 amino acid protein with a molecular load of 384 kDa. BRCA2 is structured in three main domains: the N-terminus domain, the central BRC Repeats domain and the C-terminus domain [2].

PALB2 interferes and binds the N-terminal domain of BRCA2 and contributes at the DNA binding and Rad51 D-loop formation. The central domain (BRC Repeats domain) binds free Rad51 and stabilize the complex, than facilitate the biding of Rad51 to the ssDNA. C-terminus domain interacts directly with DNA and Rad51, preventing disassembly of the already formed complex from the DNA breaks.

Several articles have shown that the C-terminus of BRCA2 is essential for proper nuclear localization of the protein, containing two NLS (Nuclear localization signal) present in the last 156 amino acids (from aminoacids 3263 - 3269 and aminoacids 3381 - 3385). Chk1 and Chk2 are well-known to phosphorylate T3387 of BRCA2, likely changing the NLS at the C-terminus and regulating BRCA2 position to the nucleus. T3387 is implicates as well in the captation and release of Rad51, with failure of phosphorylation at this site obstructing collection of Rad51 at nuclear foci. Phosphorylation of S3291 by CDK1 furthermore controls Rad51 binding to the BRCA2 C-terminus.

One of the most copiously studied and debatable C-terminal mutations of BRCA2 is the c.9976A>T transposition (rs11571833), which leads to a nonsense mutation (K3326*) in which a lysine is mutated, causing an early truncation of the protein with the loss of the last 93 amino acids. The lost domains involve Thr3387, vital for the release of Rad51, the most C-terminal nuclear localization poinand a portion of the distal Rad51-ssDNA as well as the DMC1 binding domains. One such variant is. This variant has a long research history, firstly being assumed to be pathogenic, but subsequent studies on non-cancer controls leads to a reevaluation as a non-pathogenic variant. In the present, many laboratories no longer reported its incidence considering the K3326* a VUS with benign implication [2].

Recent evidence indicates that the exon 27 of BRCA2 in which the K3326* mutation occurs, may have a key role in the repair and recovery of the cell from faulty replication processes (stalled replication forks) proving an important role in the tumor formation and progression [4].

In summary, these results suggest that the K3326* mutation leads to a functional deficit and increases the susceptibility to cancer. While deeply studied and often implicated in cancer development, it remains listed as “benign” in ClinVar and Varsome.

Our patient's case is consistent with the above findings. The patient is a carrier of the K3326* mutation, with no other identified genome modifications, being detected with breast cancer and ovarian cancer as well. K3326* mutation is classified in the ClinVar and Varsome databases as a benign mutation based on its frequency, although it is a nonsense mutation leading to a protein truncation that affects the C-terminus domain of the BRCA2 gene, involved in the DNA homologous recombination repair process. Our findings suggest that the variant is not neutral for breast and ovarian cancer risk.

Our conclusion is consistent with other three reports in the literature that associate the K3326* with an increased risk of cancer in the absence of other associated factors (last one reported in February 2021) [1-3].

Regarding the apparent contradiction between the results, we consider that it is not due to a discrepancy of sequencing analysis, but consequent to the clinical interpretation, the variant being interpreted by our laboratory Personal Genetics as a VUS (variant of uncertain clinical significance)/ potentially pathogenic, based on the patient phenotype and the arguments presented above [5-7].

Our case analysis can be extended to treatment. The presence of K3326* mutation, considered as a possible pathogenic variant of BRCA2, makes the patient a possible candidate for PARP inhibitors [8, 9] (<https://www.mycancergenome.org/content/drugs/olaparib/>).

The American College of Obstetrician and Gynecologists (ACOG) and the American Cancer Society published guidelines for management of the patients with BRCA2 gene mutation [10].

Patients diagnosed with cancer as a consequence of a BRCA2 mutation (whether it is a germline or somatic pathogenic BRCA2 mutation) often present a good response to chemotherapy agents such as PARP inhibitors (Olaparid, Niraparib, Rucaparib, Talazoparib) and platinum drugs. LOH of the wild-type BRCA2 allele is usually associated with a robust clinical response to these drugs but not in every case [10].

A tumor that is classified as homologous recombination (HDR) deficient correlates with good clinical response to platinum drugs and PARP inhibitors. LOH of the wild-type BRCA2 allele may determine whether a patient is a good candidate for PARP inhibitors therapy. If K3326* will eventually be considered a pathogenic variant of BRCA2, our patient will become a good candidate for this therapy [11-13].

Conclusive our results indicate that although K3326* does not have the same clinical significance as other BRCA2 mutations located more towards the 5'-end in the gene, its impact on the risk of breast and/or ovarian cancer is not negligible. In the future, this variant may need to be included in panels testing predisposition SNPs after further studies aiming to estimate the oncological risk. If the tumor phenotype

associated with K3326* can be classified as HDR deficient, this result would provide the motivation for the use of synthetic PARP inhibitors therapy. In summary, BRCA2-VUS, whether germline or somatic, presents an ongoing problem for clinical decision-making and our findings suggest that the K3326* should be considered as a VUS (variant of uncertain clinical significance)/potentially pathogenic [14,15].

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