Functional evaluation of five BRCA2 unclassified variants identified in a Sri Lankan cohort with inherited cancer syndromes using a mouse embryonic stem cell-based assay

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Abstract

Next-generation sequencing of Sri Lankan families with inherited cancer syndromes resulted in the identification of five BRCA2 variants of unknown clinical significance. Interpreting such variants poses significant challenges for both clinicians and patients. Using a mouse embryonic stem cell-based functional assay, we found I785V, N830D, and K2077N to be functionally indistinguishable from wild-type BRCA2. Specific but mild sensitivity to olaparib and reduction in homologous recombination (HR) efficiency suggest partial loss of function of the A262T variant. This variant is located in the N-terminal DNA binding domain of BRCA2 that can facilitate HR by binding to dsDNA/ssDNA junctions. P3039P is clearly pathogenic because of premature protein truncation caused by exon 23 skipping. These findings highlight the value of mouse embryonic stem cell-based assays for determining the functional significance of variants of unknown clinical significance and provide valuable information regarding risk estimation and genetic counseling of families carrying these BRCA2 variants.

Keywords: BRCA2, Classification, Functional assay, Inherited cancer, Next-generation sequencing, Variants of unknown clinical significance (VUS)

Breast cancer is the most common cancer in women and a leading cause of cancer morbidity and mortality in Sri Lanka [1]. Latest epidemiological reports indicate that breast cancer accounts for 13.1% of all cancers and 24% of all female cancers in the country [2]. These figures highlight the importance of identifying individuals at risk of breast cancer early so that appropriate management and preventive measures could be undertaken to reduce the morbidity and mortality associated with this disease. The advent of next-generation sequencing (NGS)-based genomic testing has facilitated rapid, precise genetic diagnosis and management of patients with inherited cancer syndromes.

In 2015, using the Illumina MiSeq NGS platform and an in-house developed validated bioinformatics pipeline, multi-gene cancer panel testing and clinical exome sequencing were successfully implemented at our center for the genetic evaluation of patients with inherited cancer syndromes [3, 4]. However, the implementation of NGS-based genomic testing into our routine clinical
cancer practice has simultaneously yielded a multitude of rare germline variants in cancer predisposing genes that are known as variants of unknown clinical significance (VUS). This poses significant challenges for both patients and clinicians, especially with regard to risk assessment, genetic counseling, and clinical decision making. Such variants might not contribute to risk assessment and may at times prompt anxiety and overtreatment. In this regard, the non-representation of genetic variants found in the Sri Lankan population in public databases is an additional drawback and challenge. To overcome this limitation, often times we resort to careful assessment of the three-generation pedigrees and testing the particular variant in other affected and unaffected family members, for further confirmation and to identify a clear pattern of co-segregation in the family members. However, the only means to precisely delineate the exact biological significance of these variants is through functional studies. This study aims to describe the functional assays which were conducted to determine the functional significance of five VUS identified in Sri Lankan families with inherited cancer syndromes.

We retrospectively analyzed the clinical and genetic test data of consecutive patients from families with two or more patients with inherited cancer syndromes who underwent NGS-based testing between January 2015 and December 2018 which were maintained prospectively in a database. Ethical clearance for the study was obtained from the Ethics Review Committee of the Faculty of Medicine, University of Colombo [EC-13-182]. Written informed consent was obtained from all the study participants. The genetic variants were classified using the five-class system as pathogenic, likely pathogenic, VUS, likely benign, or benign according to the lab classification criteria. This criteria relies on the guidelines of the American College of Medical Genetics and Genomics (ACMG) and the Association of Molecular Pathology [5]. All retained variants underwent thorough assessment and review of available evidence (e.g., population frequency databases, published literature, case/control and functional studies, internal co-occurrence and co-segregation data, evolutionary conservation, and in silico functional predictions) to arrive at a final variant classification. The variants in the BRCA1 and BRCA2 genes identified in this cohort are summarized in Table 1. We focused our studies on five VUS identified in the BRCA2 gene in this cohort for further investigations to determine their functional significance using a mouse embryonic stem (mES) cell-based assay. Mouse embryonic stem (mES) cell-based assays provide a simple and reliable assay to test the functional significance of BRCA2 VUS [6]. The assay is based on the observation that human BRCA2 to rescue the lethality of Brca2-deficient mES cells and the sensitivity of viable cells to various DNA damaging agents are used to evaluate the functional significance of the variants [6]. We used this approach to determine the functional significance of five unclassified BRCA2 germline variants [NM_

Table 1 Summary of BRCA1 and BRCA2 variants identified in Sri Lankan families with inherited cancer syndromes

| Variant | Amino acid change | ClinVar interpretation | Cancer types in index cases | Cancer types in family members |
|---------|------------------|-----------------------|----------------------------|-------------------------------|
| BRCA1:c.1575del | p.Gln526Lysfs | Pathogenic | Breast | Breast, ovarian, endometrial |
| BRCA1:c.3392A>G | p.Asp1131Gly | VUS | Breast | Breast |
| BRCA1:c.4120_4121delAG | p.Ser1374Terfs | Pathogenic | Breast | Breast, thyroid |
| BRCA1:c.5289delIG | p.Leu1764Terfs | Pathogenic | Breast, ovary | Breast, endometrial, ovarian, thyroid, hepatic, esophageal |
| BRCA1:c.68_69delIG | p.Glu23Valfs | Pathogenic | Ovary | Breast |
| BRCA1:c.1881_1884del | p.Ser628fs | Pathogenic | Breast | Breast, colorectal |
| BRCA2:c.784G>A | p.Ala262Thr | VUS | Breast and ovarian | Breast, thyroid, and endometrial |
| BRCA2:c.2353A>G | p.Ile785Val | VUS | Prostate | Colorectal and thyroid |
| BRCA2:c.2488A>G | p.Asn830Asp | VUS | Breast | Ovarian |
| BRCA2:c.6231G>C | p.Lys2077Asn | Likely Benign/VUS | Breast | Breast |
| BRCA2:c.9117G>A | p.Pro3039= | Pathogenic | Breast | Breast, thyroid, and endometrial |
| BRCA2:c.5727_5728insG | p.Asn1910fs | Pathogenic | Ovary | Ovarian, liver, colon, prostate |
| BRCA2:c.1296_1297delGA | p.Asn433Glnfs | Pathogenic | Breast, fallopian tube | Breast, liver, endometrial, colorectal, ovarian, esophageal |
| BRCA2:c.5576_5579delTTAA | p.Ile1859Lysfs | Pathogenic | Breast | Breast, endometrial, gastric |
| BRCA2:c.5621_5624delTTAA | p.Ile1874Argfs | Pathogenic | Breast | Breast, ovarian |
Examine effect of VUS on cell viability and sensitivity to DNA damaging agents.

**Fig. 1** (See legend on next page.)
Table 2 Sensitivity of mES cells expressing BRCA2 variants to different DNA-damaging agents

| Variants | MMS | Mitomycin C | Cisplatin | Camptothecin | Olaparib | IR |
|----------|-----|-------------|-----------|--------------|---------|----|
| A262T    | No  | No          | No        | No           | Yes (mild) | No |
| I785V    | No  | No          | No        | No           | No      | No |
| N830D    | No  | No          | No        | No           | No      | No |
| K2077N   | No  | No          | No        | No           | No      | No |
| P3039P   | NA  | NA          | NA        | NA           | NA      | NA |

NA not available, mES mouse embryonic stem cells, MMS methyl methanesulfonate, IR γ-irradiation.
preliminary studies with p.Ala262Thr variant did not reveal a defect in binding of BRCA2 to the chromatin (data not shown). Future studies will be aimed at understanding if p.Ala262Thr has any effect on RPA-dependent strand exchange ability of RAD51. A defect in RPA-dependent strand exchange will explain the specific effect of this variant on HR and olaparib sensitivity [16].

Abbreviations
BAC: Bacterial artificial chromosomes; GFP: Green fluorescent protein; HR: Homologous recombination; IR: γ-irradiation; mES: Mouse embryonic stem; MMS: Methyl methanesulfonate; NGS: Next-generation sequencing; VUS: Variants of unknown clinical significance; WT: Wild-type

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Authors’ contributions
NDS, KB, and SKS drafted the manuscript. KB, TS, SS, LC, and ES conducted the laboratory assays. VHWD critically reviewed the manuscript for important intellectual content. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate
Written, informed consent from all study participants and ethical clearance to conduct this study was obtained from the Ethics Review Committee, Faculty of Medicine, University of Colombo [EC-13-182].

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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