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Molecular characterization, homology modeling and docking studies of the R2787H missense variation in BRCA2 gene: Association with breast cancer

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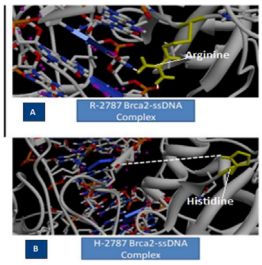
HIGHLIGHTS

- We combined bioinformatics tools and molecular ones to classify BRCA UVs.
- We used structural modeling approach to validate our assumptions.
- We were able to classify the R2787H UV as deleterious.
- Our approach could provide more efficiency for further functional study.

GRAPHICAL ABSTRACT



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ABSTRACT

The significance of many BRCA unclassified variants (UVs) has not been evaluated. Classification of these variations as neutral or pathogenic presents a significant challenge and has important implications for breast and ovarian cancer genetic counseling. Here we report a combined molecular and computational approach to classify BRCA UVs missense variations. By using the LOH (Loss of heterozygosity) analysis at the BRCA1/BRCA2 loci, five bioinformatics approaches namely fathmm, PhD-SNP, SNAP, MutationTaster and Human Splicing Finder and the association with the clinico-pathological characteristics related to BRCA tumors, we were able to classify the R2787H (in BRCA2 gene) variant as pathogenic. Then, to investigate the functional role of the R2787H variation in altering BRCA2 structure, the homology model of this variant was constructed using the Rattus norvegicus BRCA2 (PDB ID: 1IYJ) as a template. The predicted model was then assessed for stereochemical quality and side chain environment. Furthermore, docking and binding free energy simulations were performed to investigate the ssDNA-BRCA2 complex interaction. Binding energy value calculation proves that this substitution affects the complex stability. Moreover, this alteration was not found in one hundred healthy controls. These findings suggest that R2787H variant could have potential functional impact. Our approach might be useful for evaluation of BRCA unclassified variants. However additional functional analyzes may provide appropriate assessment to classify such variants.

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1. Introduction

The human tumor suppressor genes BRCA1 and BRCA2 encode large proteins of 1863 and 3418 amino acids respectively. These genes are involved in several aspects of the DNA damage and cell response (Moynahan et al., 2001; Yoshida and Miki 2004). It has been reported that mutations in BRCA genes confer an increased risk of breast cancer estimated at 55–65% for BRCA1 and 45–47% for BRCA2 (Antoniou et al., 2003). Furthermore these alterations predispose to pancreas cancer and several other types of cancer. Consequently, it is beneficial to identify BRCA1/BRCA2 mutations carriers who can benefit from early intervention measures as prophylactic bilateral, salphingo-oophorectomy or mastectomy (Kauff et al., 2002).

A significant advance in our understanding of BRCA2 function was achieved through the determination of the crystal structure of a large, evolutionarily conserved, carboxyl-terminal domain of murine BRCA2 bound to the smaller DSS1 (deleted in split hand/split foot) protein (Yang et al., 2002). The study revealed that the BRCA2 C-terminal DNA-binding domain (BRCA2 CTD) folds into a multi-domain structure comprising an N-terminal helical domain followed by three successive oligonucleotide-binding (OB) domains, which are widespread protein modules that can bind ssDNA in a sequence-specific or non-specific manner. The helical and OB domains are tightly associated with each other, with significant surface area buried at their interfaces. In the structure, DSS1 does not fold into a globular domain, and its extended conformation is intimately associated with the helical and OB1 domains through a series of hydrophobic and buried charged interactions. Therefore, it seems likely that DSS1 has evolved to become an integral part of the BRCA2 architecture, as demonstrated indirectly by the observation that DSS1 is needed to produce correctly folded recombinant BRCA2 CTD (Pellegrini and Venkitaraman, 2004). According to the breast cancer information database (9http://research.nhgri.nih.gov/projects/bic/), approximately one-third of the genetic variants in BRCA1 and 50% of those described in BRCA2 are missense variants of unknown clinical significance, termed unclassified variants (UVs).

The functional impact as deleterious or benign of a large number of BRCA missense or intronic variants has not yet been determined. A comprehensive analysis of 7461 full gene sequence analyses performed by Myriad Genetic Laboratories, reported the frequency of UVs over a 3-year period (Frank et al., 2002). Among subjects without known deleterious mutations, 13% had UVs defined as missense and intronic variants whose clinical significance remains unclear. The frequency of UVs varies by ethnicity; for example within American population, 16.5% of individuals of African ancestry had UVs. The frequency of UVs in Asian, Middle Eastern, and Hispanic populations ranges from 10% to 14% (Nanda et al., 2005). However, data from our previous study reported a higher frequency of UVs, about 37% (Riahi et al., 2013). Therefore, in our population, the characterization of the impact of such variants is of great importance. The functional effects of missense variants are difficult to predict. This becomes problematic particularly when this information is required to determine a diagnosis or prognosis. For this purpose, a number of bioinformatics tools for discriminating deleterious from neutral UVs have been developed based especially on sequence conservation and biochemical properties of amino acid changes (Goldgar et al., 2004; Fleming et al., 2003; Couch et al., 2008). Some of these tools as PhD-SNP and SNAP Predict deleterious amino acid substitution based on protein sequence information (Capriotti et al., 2006; Bromberg et al., 2008). Other prediction algorithms, including MutationTaster (Schwarz et al., 2010) can predict deleterious variants in genic regions, including coding regions and splice sites. A recent method named FATHMM (Shihab et al., 2013) uses a multiple sequence alignment to identify conserved amino acid residues and a hidden Markov model to identify amino acid substitution likely to be deleterious. Furthermore to improve predictions, FATHMM calculates pathogenicity scores that are derived from disease databases. To identify potential splicing mutations, various bioinformatics tools have been developed such as Human Splicing Finder; this tool uses position weight matrix, which represents the probability of each nucleotide occurring at each position in the binding site, to predict the effect of different alleles on splicing motifs (Desmet et al., 2009).

In this study, in order to provide an appropriate assessment to classify BRCA UVs variants, we integrated the prediction results of five bioinformatics approaches with a combination of the LOH analysis, homology modeling, docking studies, and the association with the clinico-pathological characteristics related to BRCA tumors.

2. Material and methods

2.1. Molecular investigation

This study was carried out in collaboration with the Salah Azaiz Cancer Institute, Tunis and the Department of Hereditary and Congenital Disorders, Charles Nicolle Hospital, Tunis, Tunisia. Prior to the genetic test, an informed consent was obtained from all patients and approved by the Ethical Committee of the institute. Molecular investigation and analysis were performed in the Human Genetics Laboratory, medical school of Tunis. As a first step we estimate the convenience of LOH approach to classify BRCA UVs variations. To address this matter, we assessed the incidence of LOH at the BRCA loci in familial breast tumors. We performed LOH analysis in 48 high-risk breast and ovarian cancer families who had been previously screened for the BRCA1/2 genes (Riahi et al., 2013); 12 patients (25%) harbored deleterious mutations (8 in BRCA1 and 4 in BRCA2), 13 carried an unclassified variant in either of the BRCA1/2 genes and 30 were heterozygous for any common polymorphism in these genes. The same, the rate of spontaneous LOH at the BRCA loci in the familial cases not associated with these genes but heterozygous for a polymorphism in BRCA1 (11 cases) or BRCA2 (7 cases) was determined.

2.1.1. Tumor samples and DNA extraction

We have studied 48 breast tumors. In 31 cases, frozen tumor samples were collected immediately after surgical excision and conserved in Allprotect Tissue Reagent (Qiagen) for stabilization of DNA, RNA, and proteins, then stored at −80 °C until extraction, in the remaining 17 cases DNA was extracted from paraffin-embedded tissues. Histological examinations were reviewed by tow pathologists to evaluate the percentage of tumor cells comprising these samples. Only samples containing at least 95% of tumor cells were included. Genomic DNA was extracted from peripheral blood leukocytes by standard method with proteinase K. For DNA extraction from frozen tumors and paraffin-embedded tissue, we used QIAamp DNA Mini Kit (Qiagen, Chatsworth, CA) and QIAamp DNA FFPE Tissue Kit respectively (Qiagen, Chatsworth, CA) according to the manufacturer's protocols. PCR reactions were performed on each tumor/normal pair on three highly polymorphic microsatellite markers from chromosome 13 for BRCA2 and five from chromosome 17 for BRCA1(Table 1). All PCRs were carried out using 50 ng of DNA in a total reaction volume of 25 ul containing 1X PCR buffer and 0.5 unit of Taq DNA polymerase (Promega, Madison, WI, USA), 200 mM dNTP, 16 pmol of each primer and different volumes of Mgcl2 ranging from 2.5 ul to 6 ul. The same touchdown PCR program was used for all amplicons: 95 °C (10 min) and a touchdown of ten cycles of 95 °C (15 s), 65–52 °C