

Novel nonsense mutation of BRCA2 gene in a Moroccan man with familial breast cancer

Soukaina Guaoua^{1,2}, Ilham Ratbi¹, Jaber Lyahyai¹, Siham Chafai El Alaoui², Fatima-Zahra Laarabi², Abdelaziz Sefiani^{1,2}

1. Centre de génomique humaine, Faculté de médecine et de pharmacie, Université

Mohammed V Souissi, Rabat, Morocco

2. Département de génétique médicale, Institut National d'Hygiène, Rabat, Morocco

Abstract

Background: Breast cancer is the most common cancer in women worldwide. About 5 to 10% of cases are due to an inherited predisposition in two major genes, *BRCA1* and *BRCA2*, transmitted as an autosomal dominant form. Male breast cancer is rare and is mainly due to *BRCA2* than *BRCA1* germline mutations.

Objective: Molecular study of *BRCA2* gene in man with familial breast cancer.

Methods: PCR and direct sequencing of *BRCA2* gene.

Results: Identification of novel heterozygous germline mutation c.6428C>A ; p.Ser2143Stop of *BRCA2* gene.

Keywords: male, breast cancer, *BRCA2* gene, mutation, genetic counseling.

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Introduction

Breast cancer is the most common cancer among women, accounting for about 30% of all cancers [1]. The majority are sporadic, where as 5 to 10% are due to an inherited predisposition to breast and ovarian cancers, transmitted as an autosomal dominant form with incomplete penetrance [2, 3]. Germline mutations of *BRCA1* and *BRCA2* genes are involved in nearly 10% of ovarian cancers and 3-5% of breast cancers respectively [4, 5]. The two genes belong to a class of tumor suppressor genes that maintain genomic integrity to prevent uncontrolled proliferation of tumor cells. They are also involved in DNA damage recognition, double-strand break repair, checkpoint control, transcription regulation and chromatin remodeling [6]. *BRCA1* and *BRCA2* are large genes containing 5,592 and 11,385 nucleotides spread over approximately 100,000 bases of genomic DNA each [7]. More than 1880 BRCA mutations are reported [8]. These mutations are distributed throughout the coding region and flanking intronic sequences, most are frameshifts causing

a frameshift reading of nonsense mutations or splice site alterations that lead to truncated proteins [9]. Breast cancer is a very rare disease in men, accounting for less than 1% of all cancers [10]. *BRCA2* mutations account for a significant proportion in both man breast cancer cases with or without a family history of the disease [11, 12].

We report here a novel nonsense mutation of *BRCA2* gene in a Moroccan man with breast cancer and family history of the disease.

Case report

A 63 years-old man with breast cancer was referred for genetic counselling. The family had five other women with breast cancer, and three cases with prostate, liver and uterus cancers (Figure 1).

One year before, he was diagnosed with infiltrating ductal carcinoma grade 2 of 18 mm diameter in his left breast. Hercept test (HER2) was negative, oestrogen and progesterone receptors were positive. The patient had no other associated diseases. Conservative tumorectomy was performed. He was treated by chemotherapy and local radiotherapy. Written informed consent was obtained from the patient prior to implementation of the genetic study reported here.

Genomic DNA was extracted from peripheral blood lymphocytes using salt extraction methods [13]. We chose to analyse firstly the exon 11 of *BRCA2* gene by bi-directional sequencing, because it's the longest exon with the most reported mutations BIC (Breast Cancer Information Core): <http://research.nhgri.nih.gov/bic/>. Sequence variation was verified in a new

Corresponding author:

Soukaina Guaoua

Département de génétique médicale
Institut National d'Hygiène, Avenue
Ibn Batouta, B.P. 769 Rabat – Morocco.

Tel. +212 (0) 675283238.

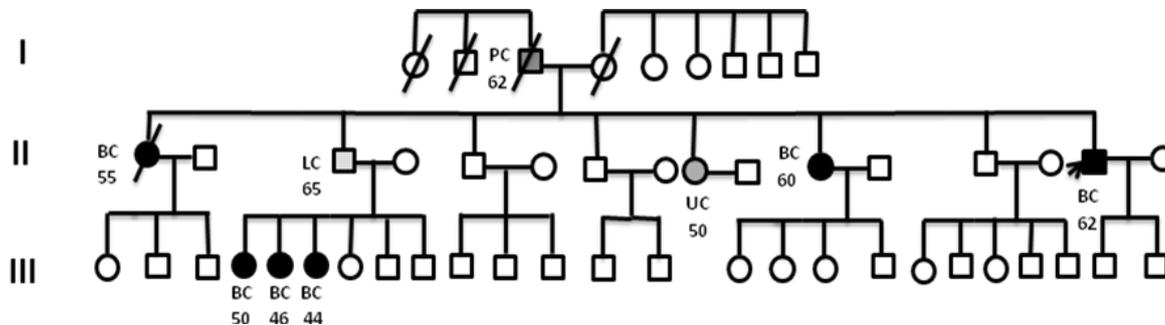
Fax. +212 (0) 537 77 20 67.

E-mail: soukainaguaoua@gmail.com

blood sample. The *BRCA2* mutation was numbered according to GenBank accession number NM_000059, in which A in the AUG start codon has number 229. In silico prediction of the functional consequence of the

nonsense variant was performed using, MutationTaster (<http://www.mutationtaster.org/>) [14] and SIFT (Sorting Intolerant From Tolerant): <http://blocks.fhcr.org/sift/SIFT.html> [15].

Figure 1: Family pedigree. Breast (BC), uterus (UC), prostate (PC) and liver (LC) cancers are indicated as well as the age at diagnosis. Diagonal slash indicates deceased, while the proband is indicated with an arrow.



Results and discussion

Molecular analysis in the patient identified a heterozygous *BRCA2* nucleotide mutation c.6428 C>A in exon 11. This variation introduces a nonsense mutation changing amino acid 2143 from serin to stop codon (Figure 2).

To our best knowledge, this mutation was never reported before in the Breast Cancer information Core database (BIC; <http://research.nhgri.nih.gov/bic/>), or other resources. The two protein prediction programmes used to predict the functional consequence of the *BRCA2* p.Ser2143Stop mutation estimated it to be pathogenic [14,15].

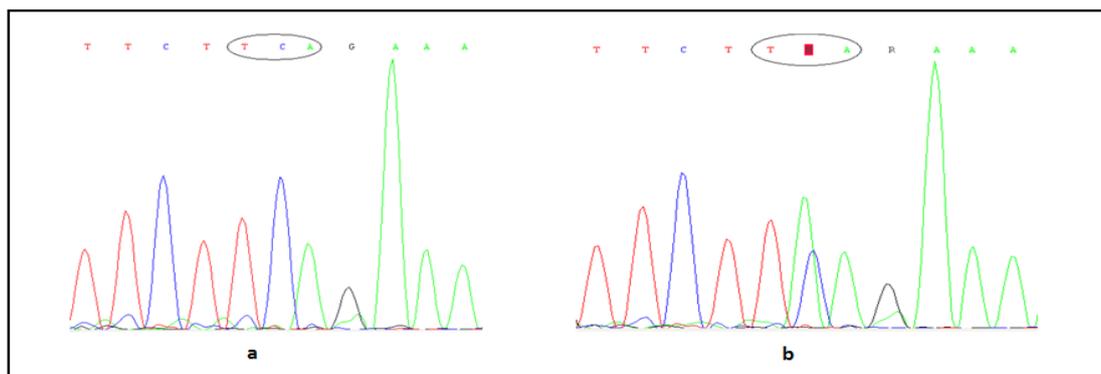
Hereditary breast and ovarian cancer syndrome is an autosomal dominant inherited cancer-susceptibility syndrome. This syndrome is characterised by multiple family members with breast or ovarian cancer or both, the presence of both breast and ovarian cancer in a single individual, and early age of breast cancer onset [16]. Approximately 10% of cases of ovarian cancer and 3-5% of cases of breast cancer are known to be associated with germline mutations in *BRCA1* and *BRCA2* [4, 5]. Male breast cancer is rare, with the peak age of onset at 71 years. *BRCA2* mutations are more frequent than *BRCA1* with 15-20% of cases giving a family history [17]. A male *BRCA2* carrier has a 6% lifetime risk of developing the disease, compared with 0.1% in the normal population [18].

Recently in Morocco, our group and others local genetic centers, have developed oncogenetic consultation for familial forms of breast and ovarian cancers. These

centers offer currently genetic testing for *BRCA1* and *BRCA2* genes, and mutational profile of these genes is becoming better known in Moroccan population [19, 20]. Even more, recently some healthy Moroccan females with a high risk of developing breast cancer benefited from presymptomatic diagnosis for a preventive management [19]. In Morocco, there is a national program against cancer that does not include actually a national genetic counseling and testing program for the familial mutations and polymorphisms which are common among the Moroccan population.

In this study, we have identified a novel nonsense germline mutation of *BRCA2* gene in a man with breast cancer and family history. This mutation is considered to be deleterious using in silico analysis. In practice for this family, we are enabled to offer a genetic counselling and to conduct DNA testing for presymptomatic diagnosis in healthy major men and women at risk if they will request it. This will allow us to introduce supervision of asymptomatic carriers in order to prevent and early diagnosis breast cancer in this family. More generally, the identification of this new mutation will allow to enrich the Moroccan Human Mutation Database that lists mutations reported in the Moroccan population (MoHuMuDa; <http://www.sante.gov.ma/Departements/INH/MoHuMuDa/index.htm>). This database was build up in 2007, and is devoted to the collection of reported human mutations in Mendelian diseases identified in the native Moroccan population, or in patients from Moroccan origin living abroad. For each mutation disease, a specific table precise the name of the gene,

Figure 2: Electropherogram showing normal sequence (a) and the heterozygous c.6428 C>A of BRCA2 gene causing the p.Ser2143Stop amino acid change (b).



the OMIM number of the disease, the published DNA, and amino-acid change, the proper nomenclature, the number of chromosomes, the frequency of the mutation, and the source of the data [21].

These mutations data could help, if recurrent mutations in Moroccan patients are identified, to establish low cost public health strategies for molecular diagnosis of patients with hereditary breast cancer. The beneficial impact on the care and counseling of individuals at risk is major, in order to reduce breast cancer mortality.

In summary, we here report a novel nonsense mutation of *BRCA2* gene in Moroccan case of a man with familial breast cancer. Genetic screening is now offered to all family members.

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