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Screening for common mutations in *BRCA1* and *BRCA2* genes: interest in genetic testing of Tunisian families with breast and/or ovarian cancer

Asma Fourati[1], Marie-Michèle Louchez[2], Joelle Fournier[2], Amor Gamoudi[1], Khaled Rahal[3], Michèle-Véronique El May[1], Ahmed El May[1], Françoise Revillion[2], Jean-Philippe Peyrat[2]

[1] Institut Salah Azaiz Cancer, Department of Immuno-Histo-Cytology, place Bab-Saadoun, 1006 Tunis, Tunisia <asma.fourati@rns.tn>

[2] Oscar Lambret Cancer Centre, Laboratory of Human Molecular Biology, Lille, France

[3] Salah Azaiz Cancer Institute, Department of Surgery, Tunis, Tunisia

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Abstract. **Background.** In the Tunisian population, as yet a limited number of *BRCA1/2* germline mutations have been reported in hereditary breast and/or ovarian cancer. These mutations are located in a few exons of *BRCA1/2*. The aim of the present study was to search for these mutations in 66 unrelated patients with hereditary breast and/or ovarian cancer in order to assess the interest in such a targeted approach for genetic testing in Tunisia. **Materials and methods.** Blood specimens from the 66 Tunisian patients, with family history of breast and/or ovarian cancer, were collected at the Salah Azaiz Cancer Institute of Tunis. The exons 5, 20 and part of exon 11 of *BRCA1* as well as part of exons 10 and 11 of *BRCA2* were analyzed by Sanger sequencing. **Results.** 12 patients had deleterious mutations in the *BRCA1* or *BRCA2* genes (18%), including a novel frameshift mutation of *BRCA1* (c.3751dup; 3780insT). Four distinct *BRCA1* mutations were detected eight patients: c.5266dup (5382insC) and c.211dup (330insA) each in three patients, c.3751dup (3870insT) and c.4041_4042del (4160delAG) each in one patient. The four remaining cases all carried the same *BRCA2* mutation, c.1310_1313del (1538delAAGA). Besides these deleterious mutations, eight polymorphisms and unclassified variants were detected, one of them being never reported (*BRCA1*c.3030T>G, p.Pro1010Pro). **Conclusion.** In this study, we show that targeting relevant exons in *BRCA1* and *BRCA2* genes allows detection of a substantial percentage of mutations in the Tunisian population. Therefore such an approach may be of interest in genetic testing of high-risk breast and ovarian cancer families in Tunisia. ▲

Key words: *BRCA1*, *BRCA2*, genetic testing, hereditary breast and ovarian cancer, targeted approach, Tunisia

Introduction

Breast cancer is the most lethal gynaecologic malignancy in Tunisian women, and its incidence and associated death rates are still increasing [1]. It is characterised by an earlier age of onset than in western countries, suggesting that genetic susceptibility genes may be involved in a substantial percentage of cases [2].

About 5-10% of all breast and ovarian cancers are considered hereditary. Germline mutations in the two major susceptibility genes, *BRCA1* and *BRCA2*, account for almost half of all inherited breast and ovarian cancers. Carriers of these mutations have an increased life time risk of developing breast and ovarian cancers [3].

Identification of mutations of *BRCA1/2* is hampered by the large size of the genes and the broad spectrum of mutations. To date, approximately 3,800 distinct mutations, polymorphisms and variants of unknown significance distributed throughout the coding regions of *BRCA1* and *BRCA2* genes have been reported in the Breast Information Core (BIC) database. The mutation spectrum of *BRCA1/2* genes depends on the countries; some mutations are unique and others are specific to some ethnic or geographically groups due to founder mutations [4]. Founder effects undergo rapid expansion from a limited number of ancestors and as a consequence allow reduced genetic heterogeneity, suggesting that molecular testing would be cheaper and quicker making easier to cover all families with hereditary breast and/or ovary cancer history. Several founder mutations of the breast and ovarian susceptibility gene *BRCA1/2* have been described. For example, three founder mutations have been identified in the Ashkenazi Jewish families of eastern European ancestry [5, 6], and specific mutations have also been described in different ethnic groups [7].

As yet, in the Tunisian population, only a few studies looked for *BRCA1/2* germline mutations in hereditary breast and/or ovarian cancer patients identifying a limited number of mutations [8-10].

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The aim of the present study was to search for these mutations in 66 patients with hereditary breast and/or ovarian cancer in order to assess the interest in such a targeted approach for genetic testing in Tunisia.

Materials and methods

Patients

A total of 66 patients from Salah Azaiz Cancer Institute with a family history of breast and/or ovarian cancers were selected between 2007 and 2009. The selection criteria for the families were as follows:
– three or more cases of breast and/or ovarian cancers in relatives of the first or second degrees;
– two cases of breast cancer with one case at young age at diagnosis (< 35 years), or one case of bilateral breast cancer, or one case of male breast cancer;
– one case with both ovarian and breast cancers;
– two cases of ovarian cancer in relatives of the first or second degrees.
For each case, family pedigree was drawn including the age at diagnosis and at death.
Patients' age varied from 22 to 85 years with a median age of 45 years. Forty five index cases were women diagnosed with a site specific breast cancer, four with a bilateral breast cancer, one with both breast and ovarian cancers, 10 with an ovarian cancer, and six index cases were males with a breast cancer. A blood sample was taken from each patient and the cancer family history was assessed by a careful inquiry. All patients were given genetic counselling and gave informed consent for testing. The use of patients' DNA was approved by the ethical committee of the Salah Azaiz Institute.

DNA extraction

All samples were screened at the Laboratoire d'Oncologie Moléculaire, Centre Oscar Lambret in Lille (France). Genomic DNA was extracted from peripheral blood collected on EDTA, using the MagNa Pure Compact instrument (Roche Diagnostics, Meylan, FRANCE) and the MagNA Pure Compact Nucleic Acid Isolation Kit, according to the manufacturer's instructions.

Screening for *BRCA1* and *BRCA2* targeted mutations

The exon 5, exon 20 and exon 11 (focusing on nucleotides 2593 to 3192 and 3622 to 4215, BIC nomenclature) of *BRCA1* as well as exon 10 (focusing on nucleotides 1467 to 2137) and exon 11 (focusing on nucleotides 5546 to 6202) of *BRCA2* were PCR amplified in a total reaction volume of 5 µl containing 0.15 mM dNTP (Euromedex, Souffelweyersheim, France), 1.5 mM $MgCl_2$, 0.05 U/µl Hot Star Taq (Qiagen-France, Courtaboeuf, France), and 0.25 pmoles/L primer pairs (Sigma Proligo, France). PCR cycling program consisted in an initial denaturation at 95 °C for 15 min, followed by 30 cycles of 94 °C 30 sec, 55 °C 30 sec, 72 °C 30 sec and 72 °C 10 min. Amplicons were then purified by incubation with SAP/EXO (Ozyme, St-Quentin-en-Yvelines, France) before sequencing. The product was sequenced in forward and reverse reactions using the Applied Biosystems Big Dye Terminator kit (Life Technologies, St Aubin, France). Cycle sequencing consisted of 1 min at 96 °C and 25 cycles of 96 °C for 10 sec, 50 °C for 5 sec and 60 °C for 4 min. After purification with ABI Big Dye X Terminator kit (Life Technologies, St Aubin, France), SANGER sequencing was performed using a 3130XL automated sequencer and analysed using Seqscape V6.5 software (Life Technologies, St Aubin, France).

Results

We detected five distinct deleterious mutations in 12 unrelated patients corresponding to a frequency of 18% (12/66) (*table 1*). Four mutations were found in *BRCA1* and one in *BRCA2*. Among these mutations, three were small deletions and two were small insertions, all of them resulting in frameshifts.
Considering the *BRCA1* gene, we identified the recurrent mutation 5382insC in three probands with breast cancer corresponding to a frequency of 4.5% (3/66), two of them presented bilateral forms. The 330insA mutation was detected in three additional site specific breast cancer families (4.5%). Two other *BRCA1* gene mutations, 3870insT and 4160delAG, were detected respectively in one ovarian cancer patient and in one male with a breast cancer, with a frequency of 1.5% (1/66). It was noteworthy that five out seven (71%) breast cancer probands with deleterious mutation of *BRCA1* exhibited triple negative tumors (*table 2*). All the three cases carrying the 5382insC mutation exhibited triple negative tumours as well as two out of three (66%) cases carrying the 330insA mutation.
In the *BRCA2* gene, the 1538delAAGA mutation was the only mutation detected. It was found in three women and one male with breast cancer, corresponding to a frequency of 6% (4/66). Besides these deleterious mutations, we detected eight distinct polymorphisms and unclassified variants, six in *BRCA1* and two in *BRCA2* genes (*table 3*), including one novel silent *BRCA1* unclassified variant, p.Pro1010Pro, observed in 6% of the patients (4/66).

Discussion

The identification of germline mutations in cancer predisposing genes is still increasing in order to apply it in routine clinical practice. In breast and ovarian familial cancers, the founder effect in *BRCA1/2* mutations remains the important way to decrease genetic variability and consequently genotyping cost and time [11].
In the present paper, we screened 66 patients with high risk of breast and/or ovarian cancer, diagnosed in the main Oncology Institute in Tunisia, for *BRCA1/2* germline mutations. We focused on three exons of *BRCA1* (5, 11 and 20) and two exons of *BRCA2* (10 and 11), based on previous results of Tunisian spectrum of *BRCA1/2* mutations reported in 100 index cases [8-10]. Our findings show that a relatively small

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