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Two *BRCA1/2* founder mutations in Jews of Sephardic origin

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Abstract Founder mutations in *BRCA1/2* genes have been detected in several Jewish communities in Israel, including in Ashkenazi Jews and Jews who immigrated to Israel from Iraq, Yemen, Iran and Afghanistan. We analyzed DNA samples of patients of Sephardic origin (descendents of Jews from the Iberian Peninsula) with breast cancer (BC) and/or ovarian cancer (OC) and additional family history of these cancers. In this study we identified 2 mutations: p.A1708E in *BRCA1* and c.67 + 1G > A (IVS2 + 1G > A) in *BRCA2*, each in 3 unrelated patients. The frequency of the two mutations was 26–31% among Sephardic high risk families and about 3% among the full cohort of 177 patients of this origin who were tested in our center. Based on haplotype analysis we concluded that these mutations are most probably founder mutations in Sephardic Jews. We recommend testing the two mutations in women of Sephardic origin who apply for BRCA testing because of personal and/or family history of BC and/or OC. Furthermore, we suggest adding them to the 5 mutations included in "The Jewish panel" of *BRCA1/2* mutations that are being tested in Israel.

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Introduction

Founder mutations in *BRCA1* and *BRCA2* genes have been detected in several communities in Israel. Three founder mutations, 185delAG and 5382insC in *BRCA1* and 6174delT in *BRCA2*, are common among Ashkenazi Jews [1]. The mutation 185delAG has also been found among Jews from Iraq. The mutation 8765delAG in *BRCA2* was detected as a founder mutation in Jews of Yemenite origin [2] and another founder mutation, p.Y978X in *BRCA1*, in Jews from Iraq, Iran and Afghanistan [3]. On the other hand, no founder mutation has yet been identified in Sephardic Jews. The Sephardic community lived for centuries in the Iberian Peninsula and was expelled from Spain and Portugal during the end of the 15th century. The descendants immigrated to Israel or to America mainly from the Balkans, but also from some other European countries and North African countries [4].

In our center, those breast and/or ovarian cancer (BC and/or OC) patients who apply for *BRCA1/2* testing were usually tested for the known founder mutations, i.e., "The Jewish panel". If the test was negative, but the patients had a family history of breast and/or ovarian cancer, they were offered additional analysis of the *BRCA1/2* genes. This analysis is based on DHPLC (denaturing high pressure liquid chromatography) [5] and sequencing if an abnormal fragment is identified. Here we describe the results of screening for mutations in the *BRCA1/2* genes in families of Sephardic Jewish origin.

Methods

Patients

BRCA1/2 testing is offered to most women who apply to our cancer-genetic counseling clinic having been diagnosed with either breast or ovarian cancer. From January 2003 until May 2010, 1210 Jewish BC and/or OC patients were tested for the 5 founder Jewish mutations (185delAG, 5382insC, p.Y978X in *BRCA1* and 6174delT, 8765delAG in *BRCA2*), 784 Ashkenazi, 359 non-Ashkenazi (Middle Eastern, North Africans and Sephardic) and 84 were of mixed origin. Sixteen percent of the Ashkenazi and 5.3% of the non-Ashkenazi patients were found to be carriers of one of these mutations. Among the non-Ashkenazi, 76 (21.2%) were Sephardic. For the purpose of the study we re-tested DNA samples of additional 101 Sephardic BC and/or OC patients that had been tested before 2003 (when not all the 5 mutations were tested in every patient). The full cohort of Sephardic BC and/or OC patients was therefore 177 (16 of them were only half Sephardic), 3(1.7%) were carriers of the mutation 185delAG in *BRCA1*. The median age at diagnosis of BC among the Sephardic patients was 46 (range 20-71), 60 (34%) had a family history of BC and/or OC of at least one 3rd degree relative.

Further *BRCA1/2* analysis was discussed with women who tested negative for the 5 mutations, particularly with those who had a significant family history of BC and/or OC (according to the counseling team). This further analysis is not covered by the National Health Insurance. Sixty four Jewish patients, from various origins, have subsequently chosen to have further screening in order to look for other mutations, 16 of them were of Sephardic origin. The mean pre-test probability of these women to be carriers, as calculated by BRCAPRO model (http://www4.utsouthwestern.edu/breasthealth/cagene/) was 40% (range: 1.6%-99.7%). Following the identification of any mutation in at least 2 unrelated families from Sephardic origin, this mutation was tested in the DNA samples of the remaining 161 Sephardic BC and/or OC patients that had previously been tested for the 5 founder Jewish mutations. In order to estimate the frequency of the two mutations in BC and/or OC Sephardic patients, we calculated their frequencies in 4 risk groups: 1. All Sephardic BC and/or OC patients 2. BC patients diagnosed under the age of 50 and OC patients diagnosed at any age. 3. BC patients diagnosed under the age of 50 and OC patients diagnosed at any age, with any family history of BC and/or OC (of at least one 3rd degree relative). 4. BC patients diagnosed under the age of 50 and OC patients diagnosed at any age, with a family history of BC and/or OC of at least one 1st degree relative (categorized as high risk families).

All women have signed an informed consent form which has been approved by the ethics committee of the hospital, allowing us to use their DNA for all investigations needed to identify the hereditary component of cancer in their families.

DNA extraction:

DNA was extracted from blood samples in EDTA tubes using the Flexigene kit (QIAGEN).

DHPLC screening and sequence analysis

Mutation analysis was performed using the WAVE apparatus from Transgenomic Inc.(Omaha, NE). PCR products were subjected to DNA chromatography.

A BigDye Terminator Cycle Sequencing version 3.1 kit (PE Applied Biosystems) was used. Cycle sequencing was performed according to the manufacturer's instructions. The products from each reaction were electrophoresed in an Applied Biosystems PRISM 3100 DNA Sequencer. The sequences for each fragment were aligned to the wild-type sequences obtained from GenBank and analyzed for sequence variation using NCBI BLAST. GenBank nos. NM_007295 and NM_000059 were used as the *BRCA1*, and *BRCA2* wild-type cDNA reference sequences. Primers sequences for the PCR reactions will be given by the authors upon request.

Mutation analysis

Allele specific amplification PCR for detection of IVS2 + 1G > A was performed using the primers: IVS2 + 1G > AF 5′-GTAAGTGCATTTTGGTCTTCTGTTTT-3′ and IVS2 + 1G > AR 5′-GGTGTAATTTATAAAGTTATA TA AA ATTTGTCAATCT-3′ and control upF 5′-AGG CACATGGTTGTTATT TGT TCT CTT CTA T -3′ and control up R-5′- GAGCCTCTAAGAATCTGATTGATGA TATAG-3′ for an internal control. Amplification was done with 1 unit of AmpliTaq gold (Applied Biosystems). The PCR conditions: 94°C-20 min; 35 cycles of 94°C-30sec; 61°C-1 min; 72°C-1 min; final extension 20 min 72°C. The PCR products were run on 3% NuSieve-Agarose gel (FMC-CAMBREX) in TBE buffer, stained with ethidium bromide and visualized under UV illumination. The PCR products were 170 bp for the IVS2 + 1G > A and 600 bp for the control band.

Restriction enzyme analysis was performed for the detection of the mutation p.A1708E. Exon 18 was amplified with the primers: EX18F-5′-GGCTCTTTAGCTTC TTAGGAC-3′ and EX18R-GAGACCATTTTCCCAGC ATC. The PCR product of 350BP was cut with *Aci*I to 2