An identical novel mutation in BRCA1 and a common haplotype in familial ovarian cancer in non-Ashkenazi Jews

L Theodor1, R Bar-Sade1, A Kruglikova1, G Ben-Baruch2, S Risel3, R Shiri-Sverdlov1, G Hirsh Yechezkel4, B Modan4, MZ Papa5, G Rechavi6 and E Friedman1

1Oncogenetics Unit, Institute of Genetics, and the 2Departments of Gynecology, 3Oncology, 4Clinical Epidemiology, 5Surgery and 6Pediatric Hemato-Oncology, Chaim Sheba Medical Center, Tel-Hashomer, 52621, Israel

Summary Unique germline mutations in BRCA1 and BRCA2 account for inherited predisposition to breast and ovarian cancer in high-risk families. In Jewish high-risk individuals of Ashkenazi (east European) descent, three predominant mutations, 185delAG and 5382insC (BRCA1) and 6174delT (BRCA2), seem to account for a substantial portion of germline mutations, and two of these mutations (185delAG and 6174delT) are also found at about 1% each in the general Jewish–Ashkenazi population. We identified a novel BRCA1 mutation in two Jewish–non-Ashkenazi families with ovarian cancer: a thymidine to guanidine alteration at position 3053, resulting in substitution of tyrosine at codon 1017 for a stop codon (Tyr1017Ter). The mutation was first detected by protein truncation test (PTT) and confirmed by sequencing and a modified restriction digest assay. Allelotyping of mutation carriers using intragenic BRCA1 markers revealed that the haplotype was identical in these seemingly unrelated families. No mutation carrier was found among 118 unselected Jewish individuals of Iranian origin. Our findings suggest that this novel mutation should be incorporated into the panel of mutations analysed in high-risk families of the appropriate ethnic background, and that the repertoire of BRCA1 mutations in Jewish high-risk families may be limited, regardless of ethnic origin.

Keywords: BRCA1; ovarian cancer; protein truncation test; rapid screening test

Germline mutations in BRCA1 and BRCA2 genes presumably account for the genetic predisposition and increased risk for breast and ovarian cancer in the majority of families with inherited predisposition to these cancers (Hall et al, 1990; Easton et al, 1993; Miki et al, 1994; Wooster et al, 1995). Thus far, more than 100 germline mutations have been identified within the BRCA1 gene (Castilla et al, 1994; Szabo and King, 1995; Langston et al, 1996), as well as several dozen in BRCA2, that, by and large, are unique to each high-risk family. A notable exception are the Jewish high-risk individuals, in whom three predominant mutations, 185delAG and 5382insC (BRCA1) and 6174delT (BRCA2), seem to account for a substantial proportion of germline mutations (Abeliovich et al, 1997). Moreover, two of these predominant mutations, 185delAG and 6174delT, are also found in the general Jewish–Ashkenazi population at a surprisingly high rate of approximately 1% each; the 5382insC mutation is found at slightly lower rates (Streuwng et al, 1995; O’ddoux et al, 1996). Our previous studies show that 185delAG mutation carriers can be detected in Jewish non-Ashkenazi populations at rates approximately similar to the Ashkenazi population (Bruchim et al, manuscript submitted). Population-based studies have defined high- and low-risk subsets for developing breast and ovarian cancer, based partly on ethnic origin (IARC, 1987). In Israel, Jewish women of Ashkenazi (east European) origin are considered at high risk for developing breast and ovarian cancers over non-Ashkenazi women, who are considered to be a low-risk population (Israel Cancer Registry, 1992). Depending on the country of origin, the Jewish population is divided into Ashkenazi and non-Ashkenazi subsets. The latter group includes diverse countries of origin, such as North Africa, Iraq, Yemen, Turkey, Bulgaria and Holland. This distinction, in turn, represents the origin of the early ancestors of the Jewish people of these ethnic subgroups since the dispersion of the Jews in the diaspora circa 70AD and since the Spanish deportation in 1492 (Goodman, 1979; Motulsky, 1995).

Except for the three predominant mutations mentioned above, no other mutations have been previously reported in Jewish high-risk individuals from ovarian cancer-prone families. Here, we report the first novel mutation detected in Israeli high-risk families of non-Ashkenazi (Iranian and Afghani) origin. Additionally, we analysed mutation carriers for haplotype-sharing with intragenic BRCA1 markers and developed a rapid detection test for this specific mutation. The rapid screening test was applied for screening of the occurrence of this mutation in an unselected panel of Jewish–Iranian men and women.

MATERIALS AND METHODS

DNA isolation and polymerase chain reaction (PCR) of genomic DNA

Genomic DNA was prepared from anticoagulated blood samples as described (Miller et al, 1988). For PTT analysis, three partly overlapping fragments covering exon 11 of the BRCA1 gene were generated by PCR using 100 ng of genomic DNA. PCR protocols
and cycling profiles were performed as previously described by Hogervorst and co-workers (Hogervorst et al., 1995). Forward primers contain a T7 promoter sequence and a eukaryotic translation initiation sequence.

**Protein truncation test (PTT)**

PCR products of expected sizes were used for PTT analysis. PTT analysis was carried out by adding 200–400 ng of T7 PCR product to the TnT/T7 coupled reticulocyte lysate system (Promega, Madison, WI, USA). The synthesized protein products were separated on a 12% SDS–polyacrylamide minigel system (Bio-Rad, Richmond, CA, USA). Gels were dried and exposed to a radiographic film for 16–40 h at – 70°C or room temperature for autoradiography.

**Sequence analysis of abnormal PTT fragments**

PCR of the fragments suspected of bearing a mutation were generated, using a biotinylated primer. Biotinylated DNA fragments were immobilized onto strepavidin-coated magnetic beads (Dynal, Oslo, Norway) and denatured to produce single-stranded templates. These templates were sequenced on the solid phase, using USB Sequenase version 2 kit, with [35S]dATP, as previously described (Svavnen et al., 1989). The samples were size separated on 6% acrylamide gel at 60 W for 2 h, and then gels were dried and autoradiographed for 24–72 h.

**Rapid screening test**

Two sets of oligonucleotide primers were designed to amplify genomic DNA for the region encompassing the mutation. Each forward primer contained one base substitution to generate a restriction site within the mutated or the wild-type allele, after PCR amplification with a common reverse primer. The first primer sequence (A) was: 5’-AAA-CAT-GGA-CTT-TTA-CAA-AAC-CTA-TA-3’ (position 3027–3052 on the cDNA with a C to G substitution at position 3049). The reverse primer corresponded to position 3177–3201. PCR reaction volume was 50 µl and included 30 pmol of each primer, 0.2 units of red-hot Taq polymerase (Advanced Biotechnologies, Leatherhead, Surrey, UK), with the AB PCR buffer supplied by the manufacturer (1.5 mm magnesium chloride), and the other standard PCR constituents. Amplification was achieved using PTC 60–100 (MJ Research, Watertown, MA, USA) and the cycling profile was as follows: denaturation at 94°C for 4 min followed by 30 cycles of denaturation (94–45 s), annealing (52–1 min) and extension (72–2 min), with a final extension step of 5 min at 72°C. PCR products were analysed on 2% agarose gels to assess the specificity and success of the reaction, and were visualized with ethidium bromide. PCR products generated with primer A and the reverse primer were digested with the restriction enzyme EcoRV (Boehringer Mannheim, Mannheim, Germany), which digests only the wild-type allele but not the mutant allele. PCR products generated with primer B and the reverse primer were digested with the restriction enzyme BfiI (MBI-Fermentas, Vilnius, Lithuania), which digests only the mutant allele but not the wild-type allele. Restriction enzyme digest products were separated on 4% Metaphor agarose (FMC, Rockland, ME, USA) gels visualized with ethidium bromide.

**Haplotype analysis**

For haplotype analysis, markers intragenic to the BRCA1 gene were used: D17S855, D17S1322 and D17S1323. PCR amplification, gel electrophoresis and autoradiography were performed using standard protocols, as previously described (Berman et al., 1996).

**Population study**

One hundred and eighteen Jewish Persian-origin individuals (58 men and 60 women) were anonymously tested for the Tyr1017ter germline mutation. The individuals were previously identified and voluntarily recruited from various departments and outpatient clinics of the Sheba Medical Center, without preselection for history of cancers. All tested individuals were unrelated to each other. The study was approved by the Human Subject Ethics Committee. The Iranian ancestry of study participants was confirmed at least three generations back.

**RESULTS**

**Patients’ clinical characteristics**

In family A, of Jewish Persian origin, ovarian cancer in the index case was diagnosed at age 40 years. The patient’s mother had ovarian cancer diagnosed at age 60 years, and a maternal aunt had ovarian cancer diagnosed at age 20 years (Figure 1). In family B, an apparently unrelated Jewish family from Afghanistan, age at diagnosis of the index case was 42 years and the mother developed ovarian cancer at 52 years of age. No other known affected family members could be ascertained. These two families were analysed as part of the oncogenetics service at the Sheba Medical Center, which counsels and tests high-risk individuals. In a 2-year period, 91 families of non-Ashkenazi origin, ascertained as high risk for breast and ovarian cancer were evaluated, five were of Iranian origin and one of Afghani origin.

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Protein truncation test (PTT) and sequence analysis of index cases

DNA samples from index cases from both families were amplified by PCR using three primer pairs (A, B and C) that encompass BRCA1 exon 11 in slightly overlapping fragments, as previously described (Hogervorst et al. 1995). PCR products of the expected size were analysed by ethidium bromide stain (data not shown) and subjected to PTT analysis. Both index cases had a typical pattern of a truncating mutation within fragment B: a normal-sized protein band and a truncated, smaller size band. Direct sequencing of these abnormal fragments using biotinylated primer revealed a thymidine to guanidine substitution at position 3053 (Figure 2). This is a non-sense mutation, substituting Tyrosine at codon 1017 for a stop codon (Tyr 1017ter).

Rapid screening test and sequence confirmation

For confirmation and rapid screening of the Tyr1017ter mutation, two sets of modified PCR primers were designed for modified restriction assay (see Materials and methods). After PCR amplification and restriction digests, DNA from individuals shown to be heterozygous for the mutation by PTT and sequencing was further confirmed by digestion with EcoRV and with Bfml.

Haplotype analysis of mutation carriers with intragenic BRCA1 markers

Using three intragenic BRCA1 microsatellite markers, the allelic patterns of the Tyr1017ter mutation carriers was determined. All mutation carriers from both families displayed an identical haplotype (Figure 1). This haplotype was distinctly different from the common Ashkenazi haplotype in 185delAG mutation carriers and was not detected in any of 100 alleles tested in individuals from the general Jewish–Iranian population.

The Tyr1017ter mutation in the general Jewish–Iranian population

The occurrence of the Tyr1017ter mutation was evaluated in a panel of Jewish–Iranian men and women (n = 118), whose DNA was available through previous screening of factor XI deficiency (Shpilberg et al., 1995) and who were unselected for personal or familial history of cancer. In the two PCR variations, none of the DNA examined showed a restriction pattern suggestive of the existence of a mutant allele. We could not screen for the occurrence of this mutation in Jews of Afghan origin as no one of this ethnic origin was available for our study.

DISCUSSION

We detected a novel BRCA1 germline mutation in two apparently unrelated Jewish–Israeli families of Iranian and Afghani extraction with a history of ovarian cancer. This is the first original mutation described in Jewish high-risk individuals, in addition to the well-known predominant mutations in high-risk families and the general Jewish–Ashkenazi population, i.e. 185delAG, 5382insC (BRCA1) (Streuwing et al., 1995; Abeliovich et al., 1997) and 6174delT (BRCA2) (Oddoux et al., 1996; Abeliovich et al., 1997).

It is probable that this mutation is of pathological significance as it results in a truncated protein. In our experience at the Oncogenetics Unit at the Sheba Medical Center, and those of other oncogenetics units in Israel (Abeliovich et al., 1997), there are only four germline mutations in BRCA1 and BRCA2 that have been detected in Jewish high-risk individuals. We have not detected any individual with the 188del11 mutation that was reported by Berman and co-workers as being prevalent in women of Ashkenazi–Jewish extraction (Berman et al., 1996).

Our finding of an identical mutation in high-risk individuals of non-Ashkenazi origin may indicate that the prevalence of this mutation in non-Ashkenazi at-risk individuals should be assessed, perhaps using the rapid screening test reported herein. If prevalence data confirm that this mutation is indeed common in this ethnic subgroup, then perhaps the scope of mutation screening in high-risk families in Israel should be expanded to include this novel mutation.

The tumourous phenotype associated with this mutation includes ovarian cancer only, with no cases of breast cancer. It is of note that germline mutations occurring at the 5' two-thirds of the BRCA1 gene are associated with a higher rate of ovarian cancer, compared with the 3' third of the gene (Gayther et al., 1995). In that respect, the mutation reported herein conforms with this suggested genotype–phenotype correlation. It remains to be seen whether families of the same ethnic origin, in whom the phenotype includes breast cancer, do display this mutation.

The mutation occurred in the background of a common haplotype when markers intragenic to the BRCA1 gene were used. This finding suggests that the Tyr1017ter mutation carriers are all descendants of an ancient founder. The possibility that germline mutations in BRCA1 are associated with an as yet unspecified biological advantage can not be ruled out. Support for this notion may come from the surprisingly high prevalence of 185delAG and 6174delT mutation carriers in the Jewish–Ashkenazi population.

The mere fact that these mutations survived the selective pressure throughout multiple generations needs to be explained and not simply dismissed as representing founder effect. Indeed, non-Jewish (Berman et al., 1996) and some Jewish–non-Ashkenazi (Bruchim et al, manuscript submitted) 185delAG mutation carriers have been found to have haplotypes distinct from Ashkenazi mutation carriers. Alternatively, the selective pressure against these mutations may not play a role, as disease manifestations occur at a post-childbearing age.
The number of Jewish–Iranian patients with ovarian cancer in Israel is small; five to ten such individuals have been reported to the Israel Cancer Registry annually during the past 10 years (Israel Cancer Registry, 1992). The mutation was detected in one of five Iranian families and in the only Afghan family tested. Thus, finding an identical mutation in two families of this ethnic origin may signify that a substantial proportion of Iranian individuals at high risk for ovarian cancer may bear this mutation, as well as in other Jewish patients originating from geographically proximate areas, e.g. Iraq, India, etc.

We did not detect this germline mutation in a panel of 118 unselected men and women of Jewish–Persian origin. This finding is in contrast to the 1% rate of mutation carriers in other BRCA1 and BRCA2 mutations in the Ashkenazi population (Streuwng et al., 1995; Oddoux et al., 1996). Several interpretations should be considered: the sample size is insufficient to detect this mutation in the general population or there is a selection bias in the patients seen at our medical centre such that there is no adequate representation of the Iranian subpopulation.

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