The 185delAG BRCA1 mutation originated before the dispersion of Jews in the Diaspora and is not limited to Ashkenazim

Revital Bruchim Bar-Sade¹, Anna Kruglikova¹, Baruch Modan⁴, Eva Gak², Galit Hirsh-Yechezkel⁴, Livia Theodor¹, Ilya Novikov⁴, Ruth Gershoni-Baruch⁷, Shulamit Risel³, Moshe Z. Papa⁵, Gilad Ben-Baruch⁶ and Eitan Friedman¹,*

¹The Susanne Levy Geatner Oncogenetics Laboratory, Institutes of ²Genetics and ³Oncology, and Departments of ⁴Clinical Epidemiology, ⁵Surgery and ⁶Gynecology, Chaim Sheba Medical Center, Tel-Hashomer 52621, Israel and ⁷Genetics Institute, Rambam Medical Center, Haifa, Israel

Received September 15, 1997; Revised and Accepted February 19, 1998

The 185delAG mutation in BRCA1 is detected in Ashkenazi Jews both in familial breast and ovarian cancer and in the general population. All tested Ashkenazi mutation carriers share the same allelic pattern at the BRCA1 locus. Our previous study showed that this ‘Ashkenazi’ mutation also occurs in Iraqi Jews with a similar allelic pattern. We extended our analysis to other non-Ashkenazi subsets: 354 of Moroccan origin, 200 Yemenites and 150 Iranian Jews. Heteroduplex analysis complemented by direct DNA sequencing of abnormally migrating bands were employed. Four of Moroccan origin (1.1%) and none of the Yemenites or Iranians was a carrier of the 185delAG mutation. BRCA1 allelic patterns were determined for four of these individuals and for 12 additional non-Ashkenazi 185delAG mutation carriers who had breast/ovarian cancer. Six non-Ashkenazi individuals shared the common ‘Ashkenazi haplotype’, four had a closely related pattern, and the rest (n = 6) displayed a distinct BRCA1 allelic pattern. We conclude that the 185delAG BRCA1 mutation occurs in some non-Ashkenazi populations at rates comparable with that of Ashkenazim. The majority of Jewish 185delAG mutation carriers have a common allelic pattern, supporting the founder effect notion, but dating the mutation’s origin to an earlier date than currently estimated. However, the different allelic pattern at the BRCA1 locus even in some Jewish mutation carriers, might suggest that the mutation arose independently.

INTRODUCTION

The search for germline mutations within breast and ovarian cancer susceptibility genes has focused on specific populations at risk for developing these malignancies. Designating an individual as high risk for breast or ovarian cancer depends primarily on a positive family history of these cancers. In addition, ethnic origin is also related to cancer risk. In Israel, the majority of breast and ovarian cancers affect Ashkenazi Jews, hence regarded a high risk group, whereas the non-Ashkenazi sub-populations (Iraqis, Moroccan, Yemenites etc.) are at a lower risk: in 1993 the rate of breast cancer per 100 000 in ‘Ashkenazi’ women was 89.8, compared with 70.7 for Asian born and 55.6 for North African born (2). Similarly, ovarian cancer also affects Ashkenazi women with rates per 100 000 of 13.1 (Israeli born) and 17 (European-American born), as compared with 6.9 (Asian born) and 9.2 (African born) (3). Between 20 and 60% of Jewish Ashkenazi individuals at risk, both ovarian cancer patients and asymptomatic family members in Israel and abroad, were found to carry a common mutation, 185delAG in BRCA1 (4–6). The same mutation was also found in ~1% of the general Jewish-Ashkenazi population who were unselected for a history of cancer (4–7). Because it was predominantly detected (and sought after) in Ashkenazi Jews, this mutation has been coined the ‘Ashkenazi mutation’. Moreover, using markers intragenic to and flanking the BRCA1 region, all tested Ashkenazi mutation carriers displayed the same allelic pattern. This finding suggested a common ancestor and a founder effect (7), and enabled an estimation of the date that this mutation arose, ~46 generations ago, or about the early 1200s (8,9).

The same mutation was detected anecdotally in non-Jewish women, where it occurs on the backdrop of a different haplotype (8,9). Sporadic cases of the 185delAG germline mutation in Jewish non-Ashkenazi families with breast and ovarian cancer have previously been reported (10,11). We previously screened 600 Iraqi Jews for this mutation, males and females unselected for personal or family history of cancer, and found a mutation carrier rate in that ethnic subgroup of 0.47%, a statistically insignificant difference from the rate in Ashkenazis (0.9%) (OR = 2, 95% CI 0.53–7.55, P = 0.37) (12). Thus, we hypothesized that the 185delAG BRCA1 mutation occurs in non-Ashkenazi Jewish ethnic groups at rates similar to the Ashkenazi population, with a similar genetic background for all Jewish mutation carriers. To test this hypothesis, several hundred ethnically diverse, unrelated non-Ashkenazi Jews were tested for the presence of the 185delAG BRCA1 germline mutation. In addition, we determined the allelic pattern at the BRCA1 locus for all non-Ashkenazi mutation carriers and compared it with that of Ashkenazi mutation carriers.

*To whom correspondence should be addressed. Tel: +972 3 530 3173; Fax: +972 3 535 7308; Email: feitan@post.tau.ac.il or eitan211@netvision.net.il
Table 1. Selected characteristics of non-Ashkenazi breast/ovarian cancer patients who were 185delAG mutation carriers

<table>
<thead>
<tr>
<th>Country of origin (patient #)</th>
<th>Cancer site</th>
<th>Age at onset (years)</th>
<th>Sporadic (S)/familial (F)</th>
<th>Relatives’ cancer site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turkey (4)</td>
<td>Ovarian</td>
<td>64</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Yemen (97)</td>
<td>Ovarian</td>
<td>43</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Egypt (139)</td>
<td>Ovarian</td>
<td>42</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Syria (456)</td>
<td>Breast</td>
<td>41</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Yemen (126)</td>
<td>Ovarian</td>
<td>71</td>
<td>F</td>
<td>Aunt’s colon</td>
</tr>
<tr>
<td>Greece (130)</td>
<td>Ovarian</td>
<td>60</td>
<td>F</td>
<td>Aunt’s colon</td>
</tr>
<tr>
<td>Iran (133)</td>
<td>Ovarian</td>
<td>37</td>
<td>F</td>
<td>Aunt’s breast</td>
</tr>
<tr>
<td>Morocco (136)</td>
<td>Ovarian</td>
<td>66</td>
<td>F</td>
<td>Niece’s ovarian</td>
</tr>
<tr>
<td>Iraq (232)</td>
<td>Ovarian</td>
<td>62</td>
<td>F</td>
<td>Mother’s breast</td>
</tr>
<tr>
<td>India (447)</td>
<td>Breast</td>
<td>50</td>
<td>F</td>
<td>Sister’s ovarian</td>
</tr>
<tr>
<td>Turkey (167)</td>
<td>Breast</td>
<td>30</td>
<td>F</td>
<td>Mother’s breast</td>
</tr>
<tr>
<td>Turkey (135)</td>
<td>Breast</td>
<td>47</td>
<td>F</td>
<td>Mother’s + aunt’s breast</td>
</tr>
</tbody>
</table>

RESULTS

Characteristics of non-Ashkenazi mutation carriers from the Oncogenetics service

A total of 112 non-Ashkenazi individuals were tested at the Oncogenetics service. Of these, we identified 12 individuals who are 185delAG mutation carriers: three Turkish-origin women (of 21 Turkish-origin tested), two Yemenites (of nine tested), and one each from Iraq (of 43 tested), Morocco (of 17 tested), Greece (of three tested), Syria (of three tested), Iran (of eight tested), India (of three tested) and Egypt (of five tested). All these women had either breast (n = 4) or ovarian (n = 8) cancer. Four were sporadic cases, with no family history, and the rest had a family history of cancers, in six of breast or ovarian and in two of colon cancer. The age range for disease diagnosis was 30–71 years. The clinical details of these individuals and their family cancer histories are summarized in Table 1.

Detection of 185delAG BRCA1 mutation in non-Ashkenazi populations. DNA samples of 354 Israeli Jews of Moroccan origin, 200 of Yemenite origin and 150 of Iranian extraction were screened for the presence of 185delAG BRCA1 germline mutation. These populations were previously studied with respect to Factor XI deficiency (13), and their authentic origins were ascertained two generations back. The mutation was detected in 4/354 (1.1%) of the Moroccan Jews and in none of the Yemenite or the Iranian Jews (Fig. 1). The rate in Moroccan Jews was not significantly different from that of Ashkenazis (0.9%) (5) (OR = 0.82, 95% CI 0.25–2.75, P = 0.75).

BRCA1 locus allelic patterns of 185delAG non-Ashkenazi mutation carriers. Comparative allelic pattern analysis was performed in all non-Ashkenazi mutation carriers (n = 16), and compared with six Jewish Ashkenazi 185delAG mutation carriers, using three intragenic BRCA1 markers and two flanking markers. All six ‘Ashkenazi’ mutation carriers were ovarian cancer patients, from the Oncogenetics service, and the age range for disease diagnosis was 42–65 years. All the Ashkenazi patients shared a common allelic pattern with all five markers (Fig. 2A). One of 16 Ashkenazi patients, all with breast cancer and none a carrier of the 185delAG or 5382insC mutations, displayed the same allelic pattern (data not shown). Three of five Moroccan-origin, one of the two Turkish-origin, the one Iraqi-origin and the Egyptian-born patient (n = 6) share the common Ashkenazi pattern with the three intragenic markers and five differ by only one extragenic marker from the common Ashkenazi pattern (Fig. 2). Four additional mutation carriers (two Moroccans, one each from Yemen and Turkey), have an allelic pattern that differs from the ‘Ashkenazi’ pattern by one intragenic marker and an additional extragenic marker (Fig. 2), a phenomenon which probably represents the instability of the dinucleotide repeat, but basically conforms with the common ‘Ashkenazi’ pattern. The remaining non-Ashkenazi mutation carriers (one each from Yemen, Turkey, Greece, Iran, Syria and India: six in all) displayed a distinct unique haplotype that was not common to any patient (Fig. 2).
Figure 2. Haplotype analysis of all tested mutation carriers using three intragenic and two flanking BRCA1 markers (indicated on the right, in their linear order). (A) Ashkenazi mutation carriers. The common Ashkenazi haplotype is shown in bold. (B) Non-Ashkenazi mutation carriers with an identical intragenic Ashkenazi haplotype: Eg, Egypt; M, Morocco; I, Iraq; Tu, Turkey. (C) Non-Ashkenazis with a similar haplotype: Ye, Yemen. (D) Non-Ashkenazi mutation carriers with a distinct, dissimilar haplotype: Ir, Iran; Ind, India.

Discussion

This study further supports the notion that the 185delAG BRCA1 germline mutation, commonly referred to as the ‘Ashkenazi mutation’, is not restricted to this particular ethnic subgroup. Rather, it is present in other Jewish ethnic subgroups, both in individuals with breast and ovarian cancers and in the general population. Moreover, in Iraqi Jews (12) and in Moroccan Jews, the population rates are in the range 0.5–1%, a statistically insignificant difference from that of the Ashkenazi Jews (5). The age standardized rate (ASR) for breast cancer in Moroccan-Jewish women is 65.6/100 000, as compared with ASR of 90.6/100 000 in women born in America and Europe (referred to as Ashkenazis), a statistically significant difference ($P = 0.005$) (2).

The observed ethnic diversity in cancer rates among Jewish women is probably accounted for by non-genetic factors, since the estimated contribution of germline mutations in cancer susceptibility genes to cancer occurrence in the general population is limited. However, the similar rates of the 185delAG BRCA1 mutation in populations with different cancer rates may facilitate the identification of the environmental factors and possibly the modifier genes involved in the pathogenesis of breast and ovarian cancer in the Jewish population.

Even though the same mutation was detected in two Yemenite and one Iranian Jewish individuals, we did not detect this mutation in the general population of Yemenite- or Iranian-born Jewish individuals. This may be due to the relatively small number of tested individuals, or might reflect a lower rate of this mutation in these ethnic subgroups. It is clear, however, that in Jewish patients with breast or ovarian cancer, who have a family history of these cancers, the 185delAG mutation can be detected, regardless of ethnic origin.

Three types of allelic patterns were detected in the 16 non-Ashkenazi mutation carriers: six (37.5%) shared the common ‘Ashkenazi haplotype’ with intragenic markers (Fig. 2), and an additional four (25%) had a pattern that was only marginally different with these same markers. Extending the allelic pattern determination to flanking markers did not significantly alter the results: the former group had either an identical pattern or a difference in a single marker, and the latter group differed by two markers, an intragenic and an extragenic. These differences in allele sizes, in particular using the extragenic marker, probably reflect an evolution of the common Ashkenazi haplotype, by way of an expansion of dinucleotide repeats, a mechanism known to occur in unstable DNA (14,15). Sharing of an identical or a closely related allelic pattern with BRCA1 markers between
Ashkenazi and a sizeable fraction (10/16: 62.5%) of non-Ashkenazi 185delAG mutation carriers, serves to support the notion of a common ancient founder for this particular mutation in Jewish people of different ethnic origin. Since the Jewish people were dispersed in the Diaspora several times throughout history and last at the time of destruction of the second temple (~70 AD), the Jewish subpopulations in East Europe (Ashkenazis), Iraq and North Africa (non-Ashkenazis) remained geographically and culturally distinct (16,17). Thus, evidence for a common ancestor in Jewish mutation carriers of different ethnic backgrounds may indicate that the common ancestor 185delAG emerged prior to the divergence of these groups during the time of the second temple, and certainly earlier than the currently estimated early 1200s (8,9).

Six of the 16 non-Ashkenazi mutation carriers (37.5%) displayed a unique allelic pattern, which differs from the common Ashkenazi pattern. This novel finding in Jewish 185delAG mutation carriers is intriguing and may stem from one of several sources. First, it might be that by using more closely spaced intragenic markers, a common allelic pattern would have been found in all Jewish individuals. However, this explanation is not consistent with the finding of allelic pattern identity over a larger chromosomal region, as was demonstrated for the majority of non-Ashkenazi mutation carriers. Alternatively, these ‘Jewish’ mutation carriers might have been offspring of non-Jewish individuals. This is unlikely, since the Jewish communities in the Diaspora, in particular in Asia and North Africa, were essentially closed societies in which intra-religious marriage rules were strictly observed. Moreover, this mutation was only rarely detected in non-Jewish individuals, despite a widespread search among diversified populations (18). This finding may signify that this particular mutation is detected so frequently because it occurs in a region of the gene prone for mutations, a mutational hot-spot. Indeed, the mutation occurs in an area of multiple adenosines, prone to DNA slippage. The fact that this mutation has survived the natural selective evolutionary pressure against it, coupled with the finding of an apparent independent focus of its rising, needs to be settled. It may indicate that this particular mutation is associated with as yet undefined biological advantage to its carriers.

MATERIALS AND METHODS

Study population

Populations screened. Individuals representing the various ethnic subgroups (Moroccan, Yemenite and Iranian Jews) 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 and their ancestry was verified at least two generations back. The study was approved by the Human Subject Ethics Committee of the Sheba Medical Center.

High risk individuals. The study also included unrelated women of all ethnic backgrounds attending the oncogenetics clinic at the Sheba Medical Center. The criteria for inclusion in this group were based on the criteria agreed on by the Israeli Oncogenetics services, which were based on the currently widely used criteria: two cases of breast or ovarian cancer in first or second degree relatives, a single case of early onset (<40 years for breast, <45 for ovarian cancer) disease or the co-occurrence of breast and ovarian cancer in the same individual in the family. All were tested and found to be carriers of the 185delAG mutation.

Genetic analysis

PCR amplified exon 2 fragments of the BRCA1 gene were generated from DNA extracted from blood samples, using primer sequences and protocol previously described by Friedman et al. (19). Heteroduplex formation was accomplished essentially as previously described (20); briefly, by denaturing 20 µl of each PCR reaction for 2 min at 94°C in the presence of denaturing solution (100 mM NaCl, 10 mM Tris–HCl, pH 7.8, 2 mM EDTA) and slow cooling to room temperature. PCR products (200 bp fragments) were loaded onto 0.6x MDE gel (Hydrolink; AT Biochem, Malvern, PA) and electrophoresed for 15 h at 50 V at room temperature in 0.6x TBE buffer. Following electrophoresis, the gels were stained with ethidium bromide. DNA sequencing was performed for each PCR fragment that consistently displayed an abnormal migration pattern on heteroduplex formation assay with the use of a biotinylated primer, as previously described (21). Haplotype analysis used markers intragenic to the BRCA1 gene: D17S855, D17S1322, D17S1323 and two additional extragenic, flanking markers: D17S1321, D17S1327. PCR amplification, gel electrophoresis and autoradiography were performed using standard protocols, as previously described (8,9).

ACKNOWLEDGEMENT

This work was performed in partial fulfilment of the requirements for the PhD degree from the Sackler School of Medicine, Tel-Aviv University, for R.B.B.-S.

REFERENCES


