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CLINICAL TRIAL

The yield of targeted genotyping for the recurring mutations in *BRCA1/2* in Israel

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Abstract
Background Hereditary breast cancer is predominantly associated with germline mutations in the *BRCA1* or *BRCA2* genes. A few recurring mutations in these genes were reported in ethnically diverse Jewish populations. Since 2013, most oncogenetic laboratories in Israel adopted a two-step approach for *BRCA1/2* genotyping, where the first step is genotyping for 14 seemingly recurring mutations—first-pass genotyping. The aim of this study was to assess the yield of this targeted *BRCA* sequencing.
Methods Clinical and genotyping data of all individuals who underwent oncogenetic counseling and first-pass *BRCA* genotyping at the Oncogenetic Service Sheba and Assaf Harofeh Medical Centers from 1 February 2013 to 30 June 2017 were reviewed. All study participants were unrelated to each other.
Results Overall, 5152 oncogenetic tests were reviewed in the present study, of which 4452 had no a priori known familial mutation. The majority of participants (68.6%) were genotyped because of personal history of cancer; 20.6% were tested because of family history of cancer, and details for the remaining 10.7% were missing. Overall, 256/4452 (5.8%) carriers were detected, 141 *BRCA1* and 115 *BRCA2* mutation carriers. In 54% of cancer-free carriers, no clinically suspicious family history of cancer was ascertained.
Conclusions The currently used scheme of first-pass genotyping in Israel seems to have a high yield of mutation detection even in the absence of a significant family history of cancer. The challenge is to optimize the currently used targeted panel of common mutations and adjust it to the accumulating new data in the Israeli population.

Keywords *BRCA1 BRCA2* germline mutations · First-pass genotyping · Unselected screening · Hereditary breast cancer

Introduction

Hereditary breast cancer (HBC) is predominantly associated with germline mutations in either the *BRCA1* (MIM# 113705) or *BRCA2* (MIM# 600185) genes. Among Jews of East European ancestry [Ashkenazi Jews (AJ)], three predominant mutations: 185delAG (HGVS c.68_69delAG) and 5382insC (c.5266dupC) in *BRCA1* and 6174delT (c.5496delT) in *BRCA2* can be detected in the majority of HBC of AJ origin. These three mutations have extensively been studied and can be detected in up to 12% of unselected AJ BC cases, 35% of unselected AJ ovarian cancer cases, with even higher rates in women diagnosed with these cancer types by age 50, and 2.5% of the general AJ population [1–3]. Over the last 20 years, a number of additional recurring mutations were described in the Israeli population, both AJ, non-Ashkenazim (non-AJ) and non-Jewish individuals.

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These include a Yemenite Jewish mutation 8765delAG (c.8537_8538delAG) in *BRCA2* [4], Asian (Persian/Iraqi/Afghani) Jewish mutation Y978* (c.8537_8538delAG) in *BRCA1* [5], a number of recurring mutations in Balkan Jews: A1708E (c.5123C>A) in *BRCA1*, R2336P (c.7007G>C) and IVS2+1G>A (c.67+1G>A) in *BRCA2* [6, 7], and North African Jewish mutation 1100delAT (c.981_982delAT) in *BRCA1* [6]. In addition, the Slavic mutations C61G (c.181T>G) and 4153delA (c.4035delA) in *BRCA1*, previously described in Russian, Polish, and Baltic populations [6], were reported amongst immigrants from the western regions of ex-Soviet Union in the early 1990s. Additional mutations, W1508X (c.4524G>A) and E720X (c.2158G>T) in *BRCA1*, were reported in a number of Muslim and Druze families, respectively [8]. The *BRCA2* mutations 4075delGT (c.3847_3848delGT) in AJ and 5164del4 (c.4936_4939delGAAA) in non-AJ were also reported infrequently but in more than one family in Israel [8]. Since 2013, most oncogenetic laboratories in Israel adopted a two-step approach for *BRCA1/2* genotyping. The first step includes a targeted panel of 14 recurring Israeli *BRCA1*/*BRCA2* mutations, and is performed in all eligible individuals following genetic counseling, regardless of ethnic origin. If this initial "first-pass genotyping" is negative, patients having 10% or higher probability of being a *BRCA* mutation carrier, as assessed by a validated risk prediction algorithm (e.g., BRCAPRO [9], Penn II [10], BOADICEA [11]), are offered full sequencing of these two genes as the second step in *BRCA* genotyping. Since the implementation of this genotyping strategy in 2013, no attempt to assess the yield of the mutations chosen for targeted sequencing in individuals referred for oncogenetic counseling in Israel was reported.

Patients and methods

Study population

The study population included all consenting individuals referred for oncogenetic counseling because of personal or family history of cancer at the Oncogenetic Services located at the Sheba Medical Center, Tel-Hashomer, and Assaf Harofeh Medical Center, between 2013 and 2017. All study participants reported herein were unrelated to each other (i.e., only one patient per family was included). The study was approved by the IRB, and each patient signed an ethically approved informed consent. Demographic and personal data including personal habits, exposures, reproductive factors, oral contraceptive use hormone replacement therapy, and detailed personal and family history of cancer were collected using a structured questionnaire from participants related to Tel-Hashomer. Self-reported ethnicity was defined by place of birth of all sets of grandparents.

DNA extraction

Peripheral blood leukocyte DNA was extracted using the PureGene kit (Gentra, Inc., Minneapolis, MN), following the manufacturer's recommended protocol.

Genotyping methodology

Samples were genotyped for the recurring *BRCA1* (c.68_69delAG, c.5266dupC, c.181T>G, c.4035delA, c.5123C>A, c.981_982delAT, c.4524G>A, c.2158G>T) and *BRCA2* (c.5946delT, c.8537_8538delAG, 4936_4939delGAAA, c.7007G>C, c.67+1G>A, c.4075delGT), using NanoChipXL (Savyon Diagnostics, Ashdod, Israel), as previously described [12]. At Assaf Harofeh, the genetic institute laboratory genotyped 13 of the above-listed mutations excluding the c.2158G>T mutation by using real-time PCR, since 2016. The mutation nomenclature was all based on HGVS (http://varnomen.hgvs.org/).

Results

Participants' characteristics

Overall, 5162 oncogenetic genotyping results were reviewed in the present study, of which 710 were performed because of a known mutation in the family. Excluding these individuals, there were 4452 eligible individuals with no a priori known familial mutation. The ethnic distribution of participants was as follows: 2012 (45.3%) AJ, 180 (4.0%) Balkan Jews (i.e., Jews from Greece, Turkey, Bulgaria, and ex-Yugoslavia), 358 (8.1%) North Africans (Jews from Morocco, Tunisia, Libya, Egypt, and Algeria), 535 (12%) Asians (Jews from Iraq, Iran, Bukhara, Syria, and Afghanistan), 437 (9.8%) mixed AJ and non-AJ, 140 (3.2%) Yemenite Jews, 88 (2%) non-Jewish (mostly Russian) Caucasians, 207 (4.7%) other mixed ancestries (i.e., Asian/North African/Balkan/non-Jewish), 10 Muslims, 9 Druze, 5 Ethiopian Jews, and 8 Jews from India (Kuchin or Bene Israel). In 463 (10.4%) cases, ancestry could not be ascertained. Relevant clinical characteristics of study participants are shown in Table 1.

BRCA1 and *BRCA2* genotyping results

Overall, 552/5162 (10.7%) genotyped individuals carried one of the 14 pathogenic mutations in either *BRCA* gene. Analysis restricted to individuals without an a priori known familial mutation yielded 256/4452 (5.8%) carriers, of whom three were double heterozygotes for *BRCA1* and *BRCA2* mutations. All but two mutations (p.W1508X, p.E720X) were detected at least once, and four mutations (p.Y978X* *BRCA1*, 8765delAG, p.R2336P, 5164del4 **BRCA2*) were

Table 1 Characteristics of patients referred to targeted testing of *BRCA1/2* (with unknown familial)

Characteristics	Patients tested, No. (%)	No. of carriers (% within ethnic group/diagnosis irrespective of ethnicity)
All patients	4452	
Female/male	3999/453	
Ethnicity		
Ashkenazi Jews (all four grandparents)	2012 (45.3)	171 (8.5)
Mixed Ashkenazi Jews	437 (9.8)	26 (5.9)
Asian Jews	535 (12)	7 (1.3)
North African Jews	358 (8.1)	2 (0.6)
Balkan Jews	180 (4.0)	7 (3.9)
Mixed non-Ashkenazi Jews	207 (4.7)	8 (3.9)
Yemenite Jews	140 (3.2)	1 (0.7)
Other (Indian, Ethiopian Jews)	13 (0.3)	0
Muslim Arabs	10 (0.2)	0
Druze	9 (0.2)	0
Other non-Jewish (European)	88 (2)	4 (4.5)
Not reported	463 (10.4)	30 (6.5)
Diagnosis		
Breast cancer	2303	109 (4.7)
Ovarian cancer	281	53 (18.9)
Breast and ovarian cancer	19	1 (5.3)
Male breast cancer	11	6 (54.5)
Pancreatic and biliary tract cancer	186	25 (13.4)
Other cancer (prostate, melanoma, gastric, colon, endometrium, lung, lymphoma, etc.)	151	8 (5.3)
Healthy	931	36 (3.9)
Inaccurate information	474	12 (2.5)

detected in more than one individual (Table 2). Notably, one of the two predominant AJ mutations in *BRCA1* (c.68_69delAG) was detected in all ethnic groups except Yemenite Jews, whereas the *BRCA2* c.5946delT mutation was also detected in individuals of North African and Balkan ancestry in addition to AJ and mixed AJ individuals (Table 2).

The majority of individuals genotyped with no a priori data on familial mutation (3058/4452, 68.7%) were tested because of personal history of cancer, additional 917 (20.6%) were referred for oncogenetic counseling and genotyping because of a family history suggestive of inherited predisposition to cancer, and for the remaining 477 (10.7%) data on the reasons for genotyping were missing. Of individuals who were cancer-free at genotyping, 422/917 (46%) had family history of ovarian cancer or male BC or BC diagnosed < 50 years of age. In this cancer-free high-risk group, 27/422 (6.4%) mutation carriers were detected, whereas 9/495 (1.8%) of the remaining healthy individuals without family history of ovarian or young-onset BC, were mutation carriers. Relevant data of 255 carriers are shown in Table 2.

Analysis restricted to 247 participants diagnosed with BC ≤ 40 years of age was subsequently carried out. For 168/247 (68%), family history was available. In this subgroup, 165 (67%) were full AJ and 33% were of mixed AJ ancestry. In this subset analysis, the overall rate of the three founder AJ *BRCA1/2* mutations (c.68_69delAG, c.5266dupC, and c.5946delT) was 31/247 (12.6%). The rates were 20.6% and 11% if all four grandparents ($n = 165$) or only two ($n = 82$) were of AJ origin, respectively. However, no other common mutations were detected in this young BC cohort. Mutation carrier rates were dependent on family history of ovarian cancer at any age or early onset (< 50) breast cancer: 6/30 (20%) if family history was present and 14/138 (10.1%) if absent (for the additional 79 patients, family history was not available).

Discussion

This study evaluated the yield of targeted *BRCA1/2* genotyping in Israeli individuals referred for oncogenetic counseling

Table 2 Characteristics of mutation carriers (unknown familial mutation)

Genes	*BRCA1*									*BRCA2*				
Mutation	185delAG	5382insC	Y978X	4153delA	C61G	A1708E	981delAT	W1508X	E720X	6174delT***	8765delAG	R2336P	IVS2+1 A->G	5164del4
No. of carriers	96	36	5	1	1	1	1	0	0	103	2	7	1	3
Ethnicity														
AJ	67	22								82				
Mixed AJ	7	8	1		1	1				7		1		
Asian Jews	2		4									1		
North African Jews							1			1				
Balkan Jews	1									1		4	1	
Mixed non-AJ	4		1								1	1		1
Non-Jewish		3		1										
Yemenite Jews											1			
Other		1												
Not reported	14		2							12				2
Diagnosis														
Breast cancer < 40 years	15	5	1							8	1	1		
Breast cancer > 40 years	21	12	1							40 (+ 4 Male BC)	1	3 (+ 1 Male BC)		1 (Male BC)
Ovarian cancer	27	8	1	1		1**	1			14				
Pancreatic cancer	10									15				
Other	1	4								3				
Not reported			2							10				2
Healthy	14	8			1					10		2	1	
Family history*	26	14	2	1	1					20	1	4	1	

* Ovarian and/or early onset (< 50) breast cancer and/or male breast cancer in first/second/third degree relatives

** Patient with metachronous breast (age 44) and ovarian (age 56) cancers

*** *One patient*—double carrier of *BRCA1* 5382insC + *BRCA2* 6174delT (breast cancer at age 49). *Two patients*—carriers of *BRCA1* 185delAG + *BRCA2* 6174delT (breast cancer at age 53, ovarian cancer at age 56)

and *BRCA1/2* genotyping, as they either had a suggestive personal or family history of cancer.

For all cases with no known mutation in the family, the yield was 256/4452 (5.8%). Of these mutation carriers, 25/1433 (1.7%) were patients of Jewish non-Ashkenazi ancestry, when 12 (48%) of them carried one of the three predominant AJ mutations, and the rest (52%) carried one of the additional genotyped mutations that were reported in non-AJ and non-Jewish populations. Four additional carriers of these non-predominant mutations were detected in 463 patients for whom ethnicity was not specified (0.9%). Of these "non-AJ" mutations, four were reported in more than one individual. One of the three predominant AJ mutations was detected in patients with unreported ethnicity at a rate of 26/463 (5.6%; Table 2), suggesting that selecting screened mutations based on self-reported ethnic origin is insufficient to determine genotyping mutation spectrum.

The results of this study show a high yield for the predominant AJ mutations in all Jewish populations in Israel, regardless of ethnicity, and a low-medium yield for the additional mutations that are included in the currently offered "universal first-pass *BRCA* genotyping" in Israel. Yet, in the non-Jewish populations, except for those of ex-Soviet Union origin, it seems that the currently included mutations result in a low yield, especially for the Druze, Indian and Ethiopian populations. One plausible reason that may have led to these results is the fact this study focused on the population primarily from central Israel, which is not representative of all the spectrum of Israeli ethnic makeup. Thus, some ethnic groups are certainly under-represented in this cohort (e.g., Druze, Muslims, Indian, and Ethiopian Jews).

Over the past few years, a number of additional seemingly recurring mutations were described in Jewish and non-Jewish patients in cancer susceptibility genes. Zick et al. reported the *BRCA1* c.224_227delAAAG mutation in two Jewish families from Kurdistan [13]. The same mutation was detected in our patients from Syria and Turkey (Bernstein-Molho and Laitman unpublished data), suggesting that this could be an additional recurring mutation in Asian Jewish population. The same group reported recently a recurring mutation c.541C>T, R181C (rs587782596) in *TP53* gene in a number of Muslim-Arab families with significant history of BC and other cancers [14]. However, targeted genotyping of more than 300 unrelated Arab high-risk individuals and sporadic breast cancer cases as well as ethnically matched cancer-free population controls did not detect this mutation [15].

An additional AJ recurring mutation S428F in the moderate cancer susceptibility gene *CHEK2* gene was previously reported in ~ 3% of Ashkenazi BC patients [16, 17].

Adding these and additional recurring mutations reported from Israel and the Middle East may enhance and optimize the yield of first-pass *BRCA* genotyping in our population. This approach was previously suggested in various populations with known "founder" mutations in *BRCA1/2* (i.e., Poland, Iceland, Russia), since knowledge of the genetic structure of particular population allows for a more rapid, less expensive and hence, more affordable first-line oncogenetic testing strategy [18].

Notably, 54% of *BRCA* mutation carriers reported herein had only limited or even absent family history of cancer, a rate similar to previously reported studies of AJ and non-Jewish populations [17, 19, 20]. These data suggest that the approach of using a targeted "first-pass genotyping," as currently practiced in Israel, is probably effective when applied to unselected population, especially for AJ patients (even for those with mixed AJ ancestry).

The prevalence of *BRCA* mutation carriers amongst unselected BC patients (full and mixed AJ diagnosed at all ages) was 109/1219 (8.9%), in line with the results reported in the literature [1, 3, 21]. Yet, the rate of the founder AJ mutations in a subset of women diagnosed under age 40 years is lower than previously reported: 11.6–20% depending on having two or four grandparents of AJ origin, compared with previously reported rates of 30% among AJ BC cases diagnosed by age 40 years [3]. The most reasonable explanation is ascertainment bias in previous studies, since only very high-risk families were initially referred for oncogenetic counseling and *BRCA* genotyping. The evolution of genetic testing over the past decade coupled with the ease of testing by using a predesigned "chip," and the increased awareness amongst patients and physicians alike, including new personalized therapeutic options like PARP-inhibitors, have all led to an increased use of genetic testing as part of the routine workup in Israeli women with breast (and ovarian) cancer in a relatively unselected manner. Therefore, our data probably reflect more realistic prevalence than reported 20 years ago.

We need to keep in mind that ~ 4% of high-risk breast and ovarian cancer individuals are reported to harbor mutations in cancer susceptibility genes other than *BRCA1/2* [22]. Rapidly decreasing costs of multi-gene NGS panels are expected to eventually replace the existing techniques. Until such a time, continued use of targeted, population-adjusted, genotyping as first-line testing seems to be a cost-effective and fast approach in unselected population, especially when deficient family history might mislead us in almost 50% of patients referred for counseling and when a population is expected to become more ethnically diverse. The challenge ahead is to optimize the currently used targeted panel of common mutations and adjust it to the accumulating new data in our population.

Conflict of interest All authors declare that they have no conflict of interest.

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