Breast Cancer Res Treat (2015) 149:223–227
DOI 10.1007/s10549-014-3218-x

EPIDEMIOLOGY

Incidence of *BRCA1* and *BRCA2* non-founder mutations in patients of Ashkenazi Jewish ancestry

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Received: 18 November 2014 / Accepted: 19 November 2014 / Published online: 6 December 2014

Abstract An estimated 1:40 individuals of Ashkenazi Jewish (AJ) ancestry carry one of three common founder mutations in *BRCA1* or *BRCA2*, resulting in the inherited cancer condition, Hereditary Breast and Ovarian Cancer (HBOC) syndrome. Targeted testing for these three mutations (*BRCA1* 187delAG, *BRCA1* 5385insC, and *BRCA2* 6174delT) is therefore recommended for all AJ breast and ovarian cancer patients, regardless of age of diagnosis or family history. Comprehensive analysis of both genes is recommended for a subset of AJ patients in whom founder mutations are not identified, but estimates of the yield from comprehensive analysis in this population vary widely. We sought to determine the proportion of non-founder mutations as a percentage of all mutations in *BRCA1* and *BRCA2* among AJ patients to inform decisions about HBOC testing strategies in this population. We analyzed the genetic testing results for 37,952 AJ patients for whom clinical testing of *BRCA1* and *BRCA2* was performed at Myriad Genetic Laboratories from January 2006 through August 2013. Analysis was limited to AJ-only patients for whom the initial test order was either (1) comprehensive testing, or (2) founder mutation testing with instructions to automatically "reflex" to comprehensive analysis if negative. Cases were excluded if a separate follow-up order was placed to reflex to comprehensive analysis only after the founder mutation testing was reported out as negative. Among all *BRCA1* and *BRCA2* mutations detected in these groups, the percentage of non-founder mutations was 13 % (104/802) and 7.2 % (198/2,769). One-hundred and eighty-nine unique non-founder mutations were detected, 76 in *BRCA1* and 113 in *BRCA2*. Non-founder mutations make up between 7.2 and 13.0 % of all *BRCA1* and *BRCA2* mutations in Ashkenazi Jews. A wide range of mutations are present, most of which are also seen in non-AJ individuals.

Keywords Hereditary Breast and Ovarian Cancer · Ashkenazi Jewish · Founder mutations · *BRCA1* · *BRCA2*

Abbreviations

AJ	Ashkenazi Jewish
HBOC	Hereditary Breast and Ovarian Cancer
HGVS	Human Genome Variation Society
LR	Large rearrangement
NCCN	National Comprehensive Cancer Network

Introduction

Pathogenic mutations in the genes *BRCA1* and *BRCA2* result in the inherited cancer condition, Hereditary Breast and Ovarian Cancer (HBOC) syndrome. Identification of individuals with HBOC has proven clinical value as it can guide the application of risk reduction strategies involving increased surveillance, chemoprevention, and/or preventative surgeries to lower risks for breast and ovarian cancer, and possibly pancreatic cancer and high-risk prostate cancer. Estimates of the prevalence of pathogenic mutations in *BRCA1* and *BRCA2* range from 1:300 to 1:500 among the world population [1–3], but higher rates are documented in some populations. Most notably, it is estimated that 2–2.5 % of individuals of Ashkenazi Jewish (AJ) ancestry carry one of three common founder mutations in the genes *BRCA1* and *BRCA2* (see Table 1) [4, 5]. The prevalence of

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these mutations in AJ women with a diagnosis of breast or ovarian cancer is estimated to be up to 10 and 41 %, respectively [6, 7]. Therefore, targeted testing for these three mutations is recommended for all AJ breast and ovarian cancer patients, regardless of age of diagnosis or family history [8].

Among AJ patients in whom a founder mutation is not identified, comprehensive analysis of both genes—full sequencing and large rearrangement analysis—is recommended in a subset of cases, depending on the strength of the personal and family history. However, the yield of comprehensive analysis in AJ patients has not been established definitively, with current estimates of the percentage of non-founder mutations ranging from 5 to 20 % [9, 10]. We sought to more definitively establish the proportion of non-founder mutations in the AJ population in order to guide decisions regarding the utility of comprehensive analysis in this population.

Methods

We compared the outcomes of clinical testing from two groups of patients. The composition of the two groups was designed to limit the analysis to patients for whom it was assured at the outset that we would have results from comprehensive analysis of *BRCA1* and *BRCA2* in a patient population for which we also knew how many founder mutations had been identified. This allowed us to determine the percentage of non-founder mutations detected as a proportion of all the mutations detected in a defined group of patients. The two groups are described below:

(1) *Comprehensive testing group (CTG)* Patients for whom the only reported ancestry was AJ and for whom the initial test ordered and performed was full sequencing of *BRCA1* and *BRCA2* with or without large rearrangement (LR) testing. Patients for whom comprehensive testing was ordered were not included in the analysis if a search of laboratory records based on patient name and date of birth found an indication of previous testing for the AJ founder mutations.

(2) *Reflex testing group (RTG)* Patients for whom the only reported ancestry was AJ and for whom the testing ordered was targeted testing for the three AJ founder mutations and "reflex" to full sequencing of *BRCA1* and *BRCA2* if no founder mutations were identified. This group was limited to cases where the reflex testing was ordered on the original test request form, and not canceled for any reason other than the detection of a founder mutation.

Table 1 Ashkenazi Jewish founder mutations in *BRCA1* and *BRCA2*

Gene	Legacy nomenclature [13]	HGVS nomenclature[a]
BRCA1	187delAG	c.68_69del (p.Glu23Valfs*17)
BRCA1	5385insC	c.5266dupC (p.Gln1756Profs*74)
BRCA2	6174delT	c.5946del (p.Ser1982Argfs*22)

[a] For a description of HGVS nomenclature, see www.hgvs.org

All testing was performed January 2006 through August 2013, and information regarding personal and family history, including ancestry, was obtained from the test request forms submitted with the samples by the ordering healthcare providers. Mutations classified as pathogenic (deleterious) and suspected pathogenic (suspected deleterious) were considered as "positive" results.

During the time period included in this analysis, a subset of patients received comprehensive LR analysis in addition to sequencing. The contribution of LRs to the findings is expected to be negligible, since LRs have previously been shown to be uncommon in patients of AJ ancestry [11, 12].

Results

There were 9,894 patients in the CTG and 28,058 in the RTG. The overall positive rate for patients with one or more *BRCA1* and *BRCA2* mutations classified as pathogenic or suspected pathogenic was 8.1 % (799/9,894) in the CTG and 9.8 % (2,750/28,058) in the RTG.

Figure 1 shows the breakdown of mutations detected in the two groups of AJ-only patients. The percentage of non-founder mutations was 13.0 % in the CTG and 7.2 % in the RTG. Twenty-two patients were found to have mutations in both *BRCA1* and *BRCA2*; 20 of these had two founder mutations, and two patients had one founder mutation and one non-founder mutation.

We detected 189 unique non-founder mutations in AJ-only patients, 76 in *BRCA1* and 113 in *BRCA2*. None of these mutations were individually common. Table 2 lists the six most common non-founder mutations identified and compares the proportions of carriers who are of full, partial, or non-AJ ancestry. Some of these mutations show evidence of being candidates for additional founder mutations in the AJ population. For example, 80 % of all carriers of *BRCA2* c.4936del report full or partial AJ ancestry. By contrast, only 6.5 % of all carriers of *BRCA2* c.3847_3848del report full or partial AJ ancestry.

Discussion

These data derived from a large clinical testing sample establish the proportion of non-founder mutations as

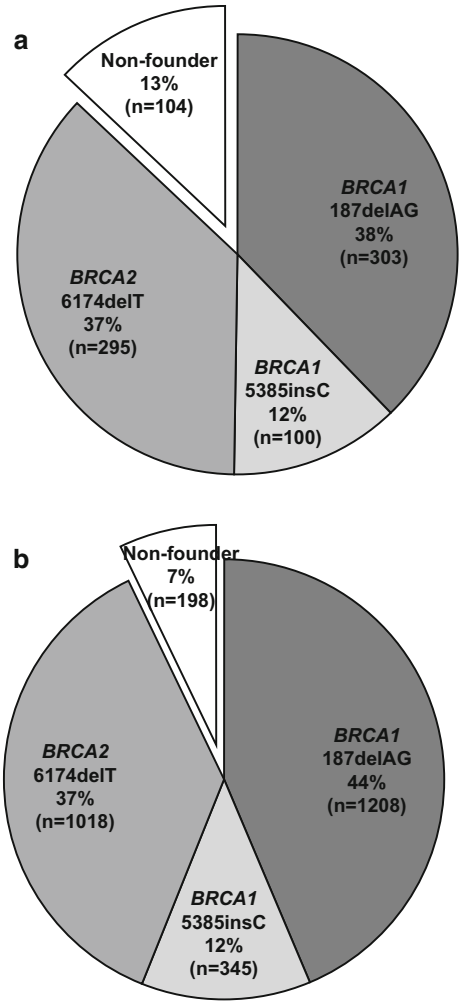

Fig. 1 Distribution of pathogenic and suspected pathogenic mutations in AJ patients for those with **a** comprehensive testing ordered as their initial test ($N = 802$) and **b** those patients for whom targeted founder mutation testing was ordered, with a request to perform full analysis if no founder mutations were detected ($N = 2{,}769$)

between 7.2 and 13 % of all *BRCA1* and *BRCA2* mutations in the AJ population in the United States. Assuming that AJ founder mutations and non-founder mutations are associated with similar cancer risks, it is possible to estimate the likelihood of finding a non-founder mutation in any AJ patient based on the estimated probability of them carrying a mutation in *BRCA1* or *BRCA2*. For example, a patient whose estimated probability of carrying a mutation is 10 % at the outset would be expected to have between a 0.7 to 1.3 % probability of carrying a non-founder mutation. In this dataset, based on the overall positive rate and the proportion of non-founder mutations identified in each group, the likelihood that an AJ patient would test positive for a non-founder mutation was 1.0 % in the CTG and 0.7 % in the RTG.

Based on the following considerations, we believe that the actual proportion of non-founder mutations in the AJ population is closest to the 7.2 % number derived from the outcomes of testing in the RTG.

(1) The number of patients in the RTG is almost threefold higher than that in the CTG, which is consistent with widely accepted practice and explicit National Comprehensive Cancer Network (NCCN) guidelines supporting the reflex strategy [8]. It should be mentioned that the relative proportion of patients in the CTG versus the RTG is an overestimate of the proportion of AJ patients for whom providers order comprehensive testing as the initial test. This is because, for the purposes of this analysis, we excluded AJ patients for whom reflex testing was not ordered, or the reflex test order was placed only after the provider received negative founder mutation results.

(2) Providers may have been more likely to order comprehensive testing as the initial test if they were more suspicious of the possibility that a patient was not of 100 % AJ ancestry. This would tend to inflate the percentage of non-founder mutations detected in this group.

(3) Some patients in the CTG may have already been known to be negative for founder mutations as a result of testing at other laboratories, research studies, or even at this laboratory under a different name or an alias. Inclusion of AJ high-risk patients already known to be negative for founder mutations could considerably inflate the percentage of non-founder mutations found in this group. Laboratory staff currently contact providers ordering comprehensive testing as an initial test for an AJ patient to inform them that the reflex testing strategy is recommended by NCCN, and required by many payers. A sampling of case notes documenting these contacts included instances where the ordering provider did mention previous founder mutation testing at other laboratories.

It should also be noted that AJ patients who carry both a founder mutation and a non-founder mutation would not usually be identified using the reflex strategy. However, only two such patients were identified in this study, indicating that this is unlikely to be a significant contributor to the results. Interestingly, one of these individuals was identified solely through founder mutation testing, as the non-founder mutation was located in the same sequencing amplicon as one of the founder mutations.

Using this data demonstrating that 7.2–13.0 % of all *BRCA1* and *BRCA2* mutations in AJ individuals are non-founder mutations, and assuming that the penetrance of AJ founder and non-founder mutations is the same, it is possible to calculate an estimated prevalence of non-founder

Table 2 Distribution of non-founder mutations in patients of Ashkenazi Jewish and other ancestries

Gene	Mutation[a]	Total (all ancestries)[b]	AJ ancestry only (%)[c]	Partial AJ ancestry (%)[d]	All or partial AJ ancestry (%)[e]
BRCA2	c.3847_3848del (4075delGT)	321	15 (4.7)	6 (1.9)	21 (6.5)
BRCA2	c.4936del (1982delA)	10	7 (70.0)	1 (10.0)	8 (80.0)
BRCA1	c.1754del (5055delG)	21	7 (33.3)	2 (9.5)	9 (42.9)
BRCA2	c.9382C>T (R3128X)	227	7 (3.1)	4 (1.8)	11 (4.8)
BRCA2	c.2808_2811del (5057delTG)	27	6 (22.2)	5 (18.5)	11 (40.7)
BRCA2	c.4829_4830del (3036del4)	460	6 (1.3)	6 (1.3)	12 (2.6)

[a] Mutations are listed with HGVS nomenclature, and the legacy nomenclature in parentheses

[b] Number of finds in patients of all ancestries for whom full sequencing was performed January 2006 through August 2013

[c] Number and percentage of all finds in patients for whom only AJ ancestry was reported

[d] Number and percentage of all finds in patients for whom AJ ancestry was reported in conjunction with one or more additional ancestries

[e] Number and percentage of all finds in patients for whom either full or partial AJ ancestry was reported

mutations in the AJ population. Based on a prevalence of 1:40 for the founder mutations in the AJ population, this comes out to 1:307–1:555, which is close to the estimated prevalence of mutations in the non-AJ population. These data support current NCCN guidelines recommending that AJ patients who test negative for founder mutations be reflexed to comprehensive analysis if their personal and family history meets testing criteria applied to non-AJ patients. The diverse selection of non-founder mutations identified, none of which were individually common, indicates that there would be little clinical utility associated with an expanded panel of mutations targeted at patients of AJ ancestry.

Finally, it should be noted that the emergence of multi-gene panels for hereditary cancer risk introduces new considerations into establishing the best strategy for appropriate follow-up testing for AJ patients who are negative for the three founder mutations in *BRCA1* and *BRCA2*. As an alternative to *BRCA1* and *BRCA2* analysis, many clinical laboratories now offer panels providing cost-effective simultaneous testing for a host of additional genes linked to inherited breast and ovarian cancer risk. The prevalence of pathogenic mutations in many of these genes is high enough that we can predict that an AJ patient is less likely to have a non-founder mutation in *BRCA1* or *BRCA2* than they are to have a significant finding in a gene like *ATM, CHEK2, PALB2*, or *TP53*. Our preliminary data from AJ patients reflexed to a multi-gene panel after negative founder mutation testing indicate that this is indeed the case (data not shown). Therefore, it is likely that the most effective strategy for those AJ patients that are candidates for testing beyond founder mutation analysis is to reflex them to an appropriate panel rather than to comprehensive analysis of *BRCA1* and *BRCA2*.

Acknowledgments The authors acknowledge Heidi McCoy and Dmitry Pruss for their contributions to this project, and all of the Myriad staff who participate in the testing process and reporting of results to providers and patients. We would also like to thank Kirstin Roundy for her assistance in formatting and editing this manuscript.

Conflict of interest The authors are full-time employees of Myriad Genetic Laboratories, Inc.

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