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SHORT REPORT

BRCA1/2 mutations and FMR1 alleles are randomly distributed: a case control study

This article has been corrected since online publication and a corrigendum also appears in this issue.

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BRCA mutation carriers were reported to display a skewed distribution of *FMR1* genotypes, predominantly within the low normal range (CGG repeat number <26). This observation led to the interpretation that *BRCA1/2* mutations are embryo-lethal, unless rescued by 'low *FMR1* alleles'. We undertook to re-explore the distribution of *FMR1* alleles subdivided into low, normal and high (<26, 26–34, and >34 CGG repeats, respectively) subgenotypes, on a cohort of 125 Ashkenazi women, carriers of a *BRCA1/2* founder mutation. Ashkenazi healthy females ($n=368$), tested in the frame of the Israeli screening population program, served as controls. *BRCA1/2* carriers and controls demonstrated a comparable and non-skewed *FMR1* subgenotype distribution. Taken together, using a homogeneous ethnic group of Ashkenazi *BRCA1/2* mutation carriers, we could not confirm the reported association between *FMR1* low genotypes and *BRCA1/2* mutations. The notion that *BRCA1/2* mutations are embryo-lethal unless rescued by the low *FMR1* subgenotypes is hereby refuted.
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INTRODUCTION

The fragile X mental retardation 1 (FMR1) gene, located on the long arm of the X chromosome, contains a repetitive CGGn trinucleotide segment in the 5′ UTR region of the gene NG_007529.1:g.5061CGG (5_55). Most individuals demonstrate 29–30 CGG triple repeats, the normal range being defined at 5–55 repeats (median $n=30$). Expansion of the CGG segment to the so-called pre-mutation range (approximately g.5061CGG (55_200)) is associated with neuro-psychiatric risks and primary ovarian insufficiency.[1] The full mutation range g.5061CGG(>200) instigates gene inactivation and loss of FMR1 protein, thereby causing fragile X syndrome, an X-linked condition, the leading cause of inherited intellectual disability in humans.[2,3]

Gleicher *et al*[4–6] have constructed a 'private' classification of subgenotypes within the normal FMR1 range (up to 55 repeats). As such, they labeled alleles of 26–34 CGG repeats g.5061CGG(26_34) as 'normal', alleles of less than 26 repeats as 'low range' g.5061CGG(5_25) and those of more than 34 repeats as 'high range' g.5061CGG(35_55). Repeats within the median range g.5061CGG(29_30) correspond allegedly with the switching point between positive and negative message and peak translation of the gene product of FMR1.[4–7] Individuals were then defined as 'normal' if both alleles were in the 'normal' range, as 'heterozygous' if one allele was outside the 'normal' range and as homozygous if both alleles were outside of the range. These genotypes were further subdivided based on whether *FMR1* alleles were above (high) or below (low) the normal range.[4–7]

This classification was used to show that young women who have 'low or high' CGG counts, whether heterozygous or homozygous, have diminished ovarian reserve.[6,7] The 29–30 CGG repeat range appears reflective of normal ovarian reserve, with higher and lower counts denoting similar risks towards premature ovarian senescence.[4–7] Ultimately, the low range allele seems to be associated with premature ovarian aging, primary ovarian insufficiency, occult primary insufficiency, premature menopause, diminished ovarian reserve, and deficient follicle recruitment and IVF outcomes.[4–7]

Other than this, carriers of *BRCA1/2* mutations, with breast cancer, were equally reported to endure primary ovarian insufficiency.[8] *BRCA1/2* mutations and *FMR1* genotypes, whether low-normal or in the permutation range, were independently reported to be associated with prematurely diminished ovarian reserve.[8] To test whether the observed effects of *BRCA1/2* and *FMR1* on ovaries were interrelated, Weghofer *et al* recently studied 99 women with *BRCA1/2* mutations and showed that they preponderantly demonstrated the low *FMR1* genotype (<26 CGG repeats) compared with the distribution displayed by a control population of 410 infertile women.[9] The authors argue that the plausible explanation for such a skewed distribution is that human *BRCA1/2* mutations are likely embryo-lethal unless rescued by a low *FMR1* subgenotype.[9] They conclude that women with *BRCA1/2*-associated breast cancer most likely carry the low *FMR1* subgenotypes and that the reported risk towards prematurely diminished ovarian reserve is FMR1-mediated.

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Using this data they generated a hypothesis maintaining that women with low alleles are expected to carry *BRCA1/2* mutations whereas those carrying normal or high CGGn *FMR1* alleles are expected to be *BRCA1/2* mutation free.[9]

In our Ashkenazi Jewish population, which is quite homogeneous, the prevalence of *BRCA1/2* founder mutations, namely c.66_67delAG (185delAG) and c.5263_5264insC (5382insC) in *BRCA1* (NM_007294.3) and c.5946delT (6174delT) in *BRCA2* (NM_000059.3), reaches 2.5%. With all of the above in mind, we undertook to re-explore the distribution of *FMR1* subgenotypes, in a cohort of Ashkenazi Jewish females, all carriers of one of the three *BRCA1/2* founder mutations.[10]

MATERIALS AND METHOD

Study population

The study was approved by the Institutional Review Board, at Rambam Health Care Campus, Haifa, Israel. Patients, at time of initial consultation, signed an informed consent, which allows for review of medical records for research purposes as long as confidentiality of the medical record is maintained. All archive files at our Oncogenetic Hereditary Breast-Ovarian Clinic were reviewed in order to identify a study cohort consisting of Ashkenazi women who carried a *BRCA1/2* mutation, whether affected ($n=97$) or non-affected ($n=28$) by cancer. *BRCA1/2* carriers were counseled and tested at our Oncogenetic Clinic between the years1995 and 2012. Ashkenazi healthy female individuals ($n=368$), with no family history of developmental impairment, were screened for fragile X syndrome, at the Bnai-Zion Medical Center, Haifa, Israel in the frame of the Israeli screening population program, and selected to have less than 55 CGG repeats, served as a control group. Among the *BRCA1/2* carriers: 61 and 19 carried the 185delAG and the 5382insC mutation in *BRCA1*, respectively; 46 had the 6174delT mutation in *BRCA2* and one participant had both a 185delAG and a 6174delT mutation. The *BRCA1/2* carrier group includes: 97 patients, of whom 66 had breast cancer, 21 had ovarian cancer and 10 had both breast and ovarian cancer; and 25 carrier females with no history of cancer. *FMR1* CGGn repeats were tested in the 125 participants from the study group.

Genotyping

FMR1 alleles. *FMR1* testing was performed by PCR amplification using previously described primers[11] with the GC-RICH PCR System (Roche Applied Science, Penzberg, Germany). Size fragment analysis was carried out by capillary electrophoresis in an ABI PRISM 3100 automatic sequencer (Applied Biosystems, Foster City, CA, USA). This methodology detects only alleles up to a maximum of 70 CGG repeats.

FMR1 data were reported for both alleles as CGGn repeats and as follows: normal range (CGG $n=26$–34), low range (less than 26 repeats), high range (between 34 and 55 repeats) and converted into subgenotypes as follows: homozygous (normal, low or high) and heterozygous (normal/low, normal/high or high/low).

BRCA1/2 ashkenazi mutations. The three predominant founder Ashkenazi mutation, namely, 185delAG and 5382insC in *BRCA1*; and 6174delT in *BRCA2*, are routinely tested in breast and/or ovarian cancer patients and family members at risk, at our oncogenetic diagnostic service at Rambam Health Care Campus, as from 1995, using RFLPs as previously described.[12,13]

Statistics. Proportions of *FMR1* subgenotypes were compared among groups using cross-tabulations and calculations of Chi-square and *t*-test statistical test when appropriate at 5% significance level. All statistical calculations were performed using SPSS 19.0 version IBM (Armonk, NY, USA).

RESULTS

The distribution of *FMR1* subgenotypes among *BRCA1/2* carriers and healthy controls was comparable (Table 1) and as follows: homozygous normal 68.0% ($n=85$) and 55.7% ($n=205$), respectively; heterozygous normal/low 22.4% ($n=28$) and 26.4% ($n=97$), respectively; heterozygous normal/high 7.2% ($n=9$) and 11.7% ($n=43$), respectively; and heterozygous high/low 1.6% ($n=2$) and 3.8% ($n=14$), respectively.

Table 2 describes the clinical characteristics of 125 *BRCA1/2* mutation carriers related to *FMR1* subgenotypes. It shows that *BRCA1/2* carriers, with at least one low allele, do not differ from those with normal or high alleles as regards morbidity, age at cancer

Table 2 Clinical profiles of 125 *BRCA1/2* mutation carriers related to *FMR1* subgenotypes

Clinical profile	*Normal or high allele,* n = *94*	*At least one low allele,* n = *31*	*Significant test (P-value)*
Site of primary cancer (n, %)			
Breast ($n=72$)	52 (55.3)	20 (64.5)	
Ovary ($n=25$)	20 (21.3)	5 (16.1)	
Asymptomatic ($n=28$)	22 (23.4)	6 (19.4)	
Total	94 (75.2)	31 (24.8)	0.663
Age of onset (M ± SD)			
Breast cancer	44.65 ± 7.62	42.7 ± 6.71	0.318
Ovarian cancer	55.25 ± 11.25	49.0 ± 7.2	0.253
Asymptomatic	44.91 ± 10.86	44.3 ± 13.7	0.914
Mutation type (n, %)			
185delAG	47 (50.0)	14 (45.2)	
5382insC	12 (12.8)	6 (19.4)	
6174delT	35 (37.2)	10 (32.3)	
5382insC/6174delT	—	1 (3.2)	
Total	94	31	0.261
BRCA1	59 (62.8)	20 (66.7)	
BRCA2	35 (37.2)	10 (33.3)	0.699
Family history (n, %)			
1st or 2nd BC-OC- PC	79 (84.0)	27 (87.1)	
Other than BC-OC- PC	10 (10.6)	3 (9.7)	
None	5 (5.3)	1 (3.2)	0.428

Abbreviations: BC, breast cancer; OC, ovarian cancer; PC, pancreatic cancer.
FMR1 normal or high CGG repeats compared with at least one low CGG repeats in relation to clinical characteristics of the 125 BRCA1/2 mutation carriers.

Table 1 *FMR1* subgenotype distribution in 125 *BRCA1/2* carriers and 368 healthy controls

Participants	*Normal/normal alleles*	*Normal/high alleles*	*High/high alleles*	*Total normal or high allele*	*Low/high alleles*	*Low/normal alleles*	*Low/low alleles*	*Total with at least one low allele*	*Total*
BRCA1/2 carriers (*n*, %)	85	9	—	94 (75.2)	2	28	1	31 (24.8)	125
Healthy controls (*n*, %)	205	43	4	252 (68.5)	14	97	5	116 (31.5)	368
Total (*n*, %)	290	52	4	346 (70.2)	16	125	6	147 (29.8)	493

FMR1 subgenotype includes: low alleles <26 CGG repeats, normal alleles between 26 and 34 CGG repeats and high alleles >34 CGG repeats.

Table 3 Comparison between *FMR1* subgenotypes of 125 *BRCA1/2* carriers and 368 healthy controls

Participants	*Normal or high allele,* n = *346*	*At least one low allele,* n = *147*	*Total,* n = *493*	*Significant test*
BRCA1/2 carriers	94 (75.2)	31 (24.8)	125 (25.4)	
Controls	252 (68.5)	116 (31.5)	368 (74.6)	$P=0.156$

FMR1 normal or high CGG repeats compared with at least one low CGG repeats in the BRCA1/2 mutation carriers and controls.

onset, type of *BRCA1/2* mutation and family history of breast–ovarian–pancreatic cancer. Most *BRCA1/2* carriers, diagnosed with breast cancer, harbored preferentially the 185delAG mutation and had a family history for hereditary breast–ovarian–pancreatic syndrome, irrespective of whether they had normal-high alleles or at least one low allele.

Table 3 details the partition of *BRCA1/2* mutations and *FMR1* subgenotypes in study and control groups. Only 24.8% ($n=31$) of *BRCA1/2* carriers exhibited one low allele comparable to 31.5% ($n=116$) of controls ($P=0.156$).

DISCUSSION

Diminished ovarian reserve has been independently reported in *BRCA1/2* mutation carriers and in women carrying low *FMR1* subgenotypes.[4–8] Weghofer *et al* hypothesized that the reported association of *BRCA1/2* mutations with premature ovarian failure is FMR1-mediated and have lately unequivocally demonstrated that *BRCA1/2* mutations are almost exclusively associated with the low *FMR1* allele.[9]

This observation was challenged by exploring the distribution of *FMR1* subgenotypes in a cohort of Ashkenazi women, carriers of a *BRCA1/2* founder mutation. We found that the *FMR1* subgenotypes were equally distributed among our *BRCA1/2* carriers of Ashkenazi descent compared with that observed in a control group from the same ethnic background.

Taken together, using a homogeneous group of Ashkenazi *BRCA1/2* mutation carriers, we could not confirm the reported association between the low *FMR1* subgenotype and *BRCA1/2* mutations. The notion that *BRCA1/2* mutations are embryo-lethal unless rescued by low *FMR1* subgenotypes is hereby refuted. *BRCA1/2* mutations were reported to be embryo-lethal only if affecting both alleles.[14] In addition, an international study conducted by us and colleagues, on 2828 *BRCA1/2* carriers, asserts that *BRCA1/2* mutation is not a risk factor for spontaneous abortions.[15] The high prevalence of *BRCA1/2* founder mutations in the Ashkenazi population does not favor the assumption that *BRCA1/2* mutation is embryo-lethal.

Lately, Brandao *et al*[16] and Ricci *et al*[17] have independently shown that the distribution of the *FMR1* subgenotypes in *BRCA* mutation carriers compares well with that obtained from non carriers, concluding that *BRCA1/2* mutations are not associated with *FMR1* low subgenotypes.

Our case control study has been conducted on relatively large cohorts, from a defined homogenous population, that harbors a limited number of *BRCA1/2* founder mutations. We can thus safely conclude that the observation asserting that women with breast cancer, carriers of a *BRCA1/2* mutation, harbor preferentially a low *FMR1* allele, is invalid.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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1 Sullivan SD, Welt C, Sherman S: FMR1 and the continuum of primary ovarian insufficiency. *Semin Reprod Med* 2011; **29**: 299–307.
2 Abrams L, Cronister A, Brown WT *et al*: Newborn, carrier, and early childhood screening recommendations for fragile X. *Pediatrics* 2012; **130**: 1126–1135.
3 Gallagher A, Hallahan B: Fragile X-associated disorders: a clinical overview. *J Neurol* 2012; **259**: 401–413.
4 Gleicher N, Weghofer A, Barad DH: A pilot study of premature ovarian senescence. I. Correlation of triple CGG repeats on the FMR1 gene to ovarian reserve parameters FSH and anti-Müllerian hormone. *Fertil Steril* 2009a; **91**: 1700–1706.
5 Gleicher N, Weghofer A, Oktay K, Barad D: Relevance of triple CGG repeats in the FMR1 gene to ovarian reserve. *Reprod Biomed Online* 2009b; **19**: 385–390.
6 Gleicher N, Weghofer A, Oktay K, Barad DH: Correlation of triple repeats on the FMR1 (fragile X) gene to ovarian reserve: a new infertility test? *Acta Obstet Gynecol Scand* 2009c; **8**: 1024–1030.
7 Gleicher N, Weghofer A, Barad DH: Ovarian reserve determinations suggest new function of FMR1 (fragile X gene) in regulating ovarian ageing. *Reprod Biomed Online* 2010; **20**: 768–775.
8 Oktay K, Kim JY, Barad D, Babayev SN: Association of BRCA1 mutations with occult primary ovarian insufficiency: a possible explanation for the link between infertility and breast/ovarian cancer risks. *J Clin Oncol* 2010; **28**: 240–244.
9 Weghofer A, Tea MK, Barad DH *et al*: BRCA1/2 Mutations Appear Embryo-Lethal Unless Rescued by Low (CGG n,26) FMR1 Sub-Genotypes: Explanation for the "BRCA Paradox"? *PLoS One* 2012; **7**: e44753.
10 King MC, Marks JH, Mandell JB: New York Breast Cancer Study Group. Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2. *Science* 2003; **302**: 643–646.
11 Mornet E, Chateau C, Hirst MC *et al*: Analysis of germline variation at the FMR1 CGG repeat shows variation in the normal-premutated borderline range. *Hum Mol Genet* 1996; **5**: 821–825.
12 Gershoni-Baruch R, Dagan E, Fried G *et al*: BRCA1 and BRCA2 founder mutations in patients with bilateral breast cancer. *Eur J Hum Genet* 1999; **7**: 833–836.
13 Rohlfs EM, Learning WG, Friedman KJ *et al*: Direct detection of mutations in the breast and ovarian cancer susceptibility gene BRCA1 by PCR-mediated site-directed mutagenesis. *Clin Chem* 1997; **43**: 24–29.
14 Konishi H, Mohseni M, Tamaki A *et al*: Mutation of a single allele of the cancer susceptibility gene BRCA1 leads to genomic instability in human breast epithelial cells. *Proc Natl Acad Sci USA* 2011; **108**: 17773–17778.
15 Friedman E, Kotsopoulos J, Lubinski J *et al*: Hereditary Breast Cancer Clinical Study Group. Spontaneous and therapeutic abortions and the risk of breast cancer among BRCA mutation carriers. *Breast Cancer Res* 2006; **8**: R15.
16 Brandão RD, van Roozendaal K, Tserpelis D, Blok MJ: FMR1 low sub-genotype does not rescue BRCA1/2-mutated human embryos and does not explain primary ovarian insufficiency among BRCA1/2-carriers. *Hum Reprod* 2013; **28**: 2308–2011.
17 Ricci MT, Pennese L, Gismondi V *et al*: The *FMR1* CGG repeat test is not a candidate prescreening tool for identifying women with a high probability of being carriers of *BRCA* mutations. *Eur J Hum Genet* 2014; **22**: 280–282.