FMRI CGG allele length in Israeli BRCA1/BRCA2 mutation carriers and the general population display distinct distribution patterns

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Summary

Premature ovarian failure and diminished ovarian reserve have been noted both in female BRCA1/BRCA2 mutation carriers and in carriers of the Fragile X syndrome FMRI gene CGG repeat size premutation. Based on the observation that BRCA mutation carriers do not harbour long CGG repeats in the FMRI gene, it was hypothesized that BRCA-associated premature ovarian failure is mediated via FMRI. To test this notion, we evaluated the distribution of constitutional FMRI genotypes in 188 BRCA1/BRCA2 mutation-positive Jewish Ashkenazi women and 15,708 female, mostly Ashkenazi controls in Israel. BRCA1/BRCA2 mutation carriers displayed a unique distribution of FMRI genotypes compared with controls ($p = 0.018$) with a prominence of the shorter CGG alleles (<26 repeats). There was no allele size distribution differences within BRCA carriers when comparing cancer free ($n = 95$) and breast cancer affected women ($n = 93$) ($p = 0.43$). In conclusion, BRCA mutation carriers exhibit a distinct CGG FMRI repeat size pattern compared with the general population, but it is unlikely to account for the reported diminished ovarian reserve or act as a modifier breast cancer gene in BRCA mutation carriers.

1. Introduction

The fragile X mental retardation 1 (FMRI) gene, is localized to Xq27.3, and contains a repetitive DNA segment, the (CGG)n trinucleotide. Mutation status depends on the number of CGG repeats, women who carry 58–200 repeats and those who carry more than 200 repeats are designated premutation and full mutation carriers of the Fragile X syndrome (MIM #300624), respectively (Willemsen et al., 2011). One of the phenotypes reportedly associated with having a long CGG repeat, is premature ovarian failure, or primary ovarian insufficiency (Wittenberger et al., 2007). Moreover, diminished ovarian reserve was also reportedly associated with a CGG repeat size that is not in the range of the “normal size repeat”, defined as 26–34 repeats (Gleicher et al., 2010a, 2010b). Diminished ovarian reserve was also noted in BRCA1/BRCA2 mutation carriers (Oktay et al., 2010). These phenotypic similarities in ovarian reserve, have led Weghofer and co-workers to analyze FMRI CGG repeat length in BRCA1 and BRCA2 mutation carriers (Weghofer et al., 2012). They report that BRCA1/BRCA2 mutation carriers almost exclusively show low CGG repeats, with significant allele distribution differences between mutation carriers and noncarrier infertile controls. They conclude that this unique FMRI CGG allele distribution in BRCA1/BRCA2 carriers underlies many of the noncancerous ovarian phenotypes reported in mutation carriers. In addition, based on their results, Weghofer and co-workers hypothesized that the unique FMRI CGG repeat alleles may underlie the so called “BRCA paradox”, where BRCA alleles in the embryo exert an anti-proliferative effect (that may account for embryonic lethality in BRCA null embryos) whereas in adult tissues lack of BRCA protein expression is associated with a strong proliferative effect that eventually

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leads to tumor formation in the relevant tissues, e.g. breast and ovarian.

The present study was performed to validate and expand this previous report, targeting Jewish Israeli BRCA1/BRCA2 mutation carriers and a large Jewish control population group.

2. Materials and methods

(i) Study participants

Individuals counselled and genotyped for BRCA1/BRCA2 germline mutations or FMR1 CGG repeat size at the Danek Gertner Institute of Human Genetics at the Sheba Medical Center, Tel-Hashomer, Israel from 1 January 2008–31 December 2012 were eligible. The Oncogenetics Unit recruited women who were counselled and genotyped for being at high risk for developing breast/ovarian cancer as determined by standard criteria (Laitman et al., 2012) and were subsequently found to harbour either a BRCA1 or BRCA2 mutation (see below). The control group was composed of women who were genotyped for their FMR1 CGG repeat length status in the context of prenatal genetic testing, offered in Israel. The study was approved by the ethics committee of the Sheba Medical Center and each participant gave an informed consent to participate in the approved study protocol.

(ii) Analysis of the predominant Jewish mutations in the BRCA1/BRCA2 genes

Mutational analyses for two of the three founder mutations (185delAG in BRCA1 and 6174delT in BRCA2) were carried out by restriction enzyme digest of PCR products, and analysis of the digested PCR products on agarose gels as previously described by us (Laitman et al., 2012). For each of these mutations, a known mutation carrier was used as a positive control in each experiment and each mutation was confirmed by sequencing.

(iii) FMR1 CGG repeat analysis

FMR1 analyses were performed as previously reported (Gleicher et al., 2011). Briefly, the FMR1 repeat region is amplified using a fluorescent primer, the PCR product will then be mixed with Liz 500 internal size standard (Applied Biosystems Inc., Foster City, CA, USA), and analyzed on the ABI Prism 3100 genotyper. The Genescan raw data were analyzed using the Genemapper software (version 3.1) to obtain the allele repeat in base pairs.

(iv) Statistical analyses

Proportions of FMR1 genotypes and sub-genotypes were compared between the two study groups using cross-tabulations and calculations of χ² and Cramer’s V statistics. When comparing (CGG)n as a continuous function between the groups, nonparametric testing was used because (CGG)n in populations tends to be positively skewed. Mann–Whitney U tests were conducted to evaluate differences between the two groups in median change in (CGG)n of both alleles. All statistical calculations were performed utilizing SPSS, version 18 (Chicago, IL, USA).

3. Results

(i) Participant characteristics

(a) BRCA1/BRCA2 mutation carriers

Overall, there were 188 mutation carriers who participated in the study, 97 carried the BRCA1*185delAG mutation and 91 carried the BRCA2*6174delT mutation. There were 48 women who were diagnosed with breast cancer who harboured the 185delAG mutation and the mean age at diagnosis was 44.05 ± 12.1 years, and 45 breast cancer cases that carried the 6174delT mutation and the mean age at diagnosis was 44 ± 12.1 years. All other BRCA1/BRCA2 mutation carriers (n = 95) were cancer free (mean age at counselling and genotyping was 44 years for BRCA1 carriers and 44-2 years for BRCA2 mutation carriers).

(b) Controls

Overall 15,708 women underwent prenatal genetic testing to define the repeat length of the CGG trinucleotide repeat in the FMR1 gene in order to evaluate the need for prenatal diagnosis of embryonic Fragile X syndrome (Toledano-Alhadef et al., 2001; Berkenstadt et al., 2007). The mean age at counselling and genotyping was 26.7 ± 4.3 (19–37) years and 59% were of Ashkenazi origin. Notably, these women did not undergo BRCA1/BRCA2 mutational analysis.

(ii) CGG FMR1 repeat length genotyping

In the control, non-BRCA mutation carrier population, one FMR1 allele repeat number range was 8–600 repeats, with a median allele size of 30 repeats, and the second allele repeat number range was 15–600 repeats (median allele repeat size 30 repeats). Notably, this FMR1 CGG repeat length distribution was similar to that reported in other, ethnically diverse populations (Gleicher et al., 2010b; Gleicher et al., 2011).

Amongst BRCA mutation carriers, one FMR1 allele repeat number range was 14–40 repeats with a median allele of 29 repeats, and the second allele repeat size range was 23–92 repeats with the median allele repeat number of 30 repeats.
In the present study, the FGMR1 CGG trinucleotide repeat length distribution among Jewish Israeli BRCA1/BRCA2 mutation carriers was different from that exhibited by the general Israeli, mostly Ashkenazi, population. These FGMR1 CGG allele distribution differences results are in line with those of Weghofer et al. (2012) who previously reported a unique FGMR1 CGG allele distribution in BRCA1/BRCA2 mutation carriers undergoing fertility treatment compared with noncarriers recruited from an infertility clinic. Yet, unlike the almost complete absence of long CGG repeat sizes (34 repeats and above) in the study by Weghofer and co-workers (2012), in the present study 8.5% of BRCA1/BRCA2 carriers had at least one long CGG allele, a rate that was not statistically different to that of the control population (12.9%). The basic premise of the Weghofer et al. (2012) study was that the long CGG allele in the presence of a BRCA mutation may be embryonic lethal, and that rescue may be conferred by the existence of short (<26 repeats) alleles; however, our results do not support this notion. In line with our previous data, smaller studies published from Israel (Dagan et al., 2014), Italy (Ricci et al., 2014) and Holland (Brandão et al., 2013) also failed to show the lack of long CGG FGMR1 alleles in BRCA mutation carriers. Another recent study that focused on ovarian cancer cases (n = 87) showed that ovarian cancer cases who are BRCA pathogenic mutation carriers (n = 15) exhibit a normal-range prevalence of FGMR1 CGG alleles, whereas ovarian cancer patients who are BRCA wild-type, display a trend (not reaching statistical significance) towards higher prevalence of low CGG repeats of the FGMR1 gene (Gleicher et al., 2014). Moreover, the data presented herein and the above mentioned previous reports (summarized in Table 3) also question the putative role attributed by Weghofer and co-workers (2012) to FGMR1 alleles in mediating premature ovarian failure in BRCA mutation carriers.

Several reasons may underlie the discrepancy in the results on the rates of the longer CGG alleles between the original Weghofer and co-workers (2012) study and the subsequent studies: the ethnicity of the genotyped groups in both Israeli studies was different, with mostly Ashkenazi Jewish women genotyped in these studies compared with mostly Caucasian European and American women in the original study. These ethnic differences are reflected in the location and type of mutations in both BRCA1 and BRCA2 genes, and given the genotype – phenotype correlations reported in BRCA1/BRCA2 mutation carriers, the Weghofer and co-workers (2012) study results may be in part attributed to these genetic and ethnic differences. This is not a valid argument to account for the lack of replication in the Italian and Dutch studies. Another factor that may have led to the discordant results is the mode of recruitment and ascertainment of BRCA mutation carriers: a cancer and prenatal genetics clinic in the present study and the study by Dagan and co-workers (2014), a familial breast cancer clinic in the study by Ricci et al. (2014) and an infertility clinic in the Weghofer et al. (2012) study. However, this latter explanation is unlikely to account.
for the inconsistent results, as previous studies reported a similar FMR1 CGG repeat length in both fertile and infertile women of diverse ethnicities (Toledano-Alhadef et al., 2001; Berkenstadt et al., 2007; Gleicher et al., 2010b; Gleicher et al., 2011).

Unique ovarian-reproductive phenotypes have previously been reported in BRCA1 and BRCA2 mutation carriers, in addition to ovarian cancer the following have been observed: an increased risk for recurrent miscarriages (Gal et al., 2004), a skewed ratio of male to female offspring (de la Hoya et al., 2003). The mechanisms for these noncancerous phenotypes and the pathways that underlie them are presently unclear. It is possible that the unique FMR1 CGG allele size, as reflected by the different allelic distribution between cases and controls in our study,
plays a role in these additional phenotypes, but currently this possibility remains speculative at best.

The data presented also make it unlikely that the FMR1 CGG repeat length size plays a significant role in affecting breast cancer penetrance in BRCA1 or BRCA2 mutation carriers, as the allele distribution was similar in breast cancer affected women and in ethnically matched cancer-free mutation carriers. This is further emphasized by the results of Gleicher and co-workers (2014) in BRCA carriers diagnosed with ovarian cancer, displaying a normal range of FMR1 CGG alleles. It is noteworthy that the present study and all previous studies are underpowered to detect any subtle effect of the FMR1 CGG genotype on BRCA1/BRCA2 penetrance.

In conclusion, the CGG trinucleotide repeat in the FMR1 gene in Jewish Israeli women, predominantly of Ashkenazi origin, shows a distinct distribution among BRCA1/BRCA2 mutation carriers compared with the general population, but these differences are unlikely to account for the common phenotype of premature ovarian failure.

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Declaration of interest
None.

References