FMRI low sub-genotype does not rescue BRCA1/2-mutated human embryos and does not explain primary ovarian insufficiency among BRCA1/2-carriers

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INTRODUCTION

BRCA1/2-mutation carriers have increased risk of predominantly breast and ovarian cancer, but also other cancers. To prevent transmission of mutations to their offspring, nowadays, BRCA1/2-mutation carriers can opt for IVF with preimplantation genetic diagnosis (PGD). Oktay et al. reported that BRCA1-mutation carriers subjected to ovarian stimulation produce lower number of mature oocytes than non-carriers (Oktay et al., 2010; Titus et al., 2013). Consequently, the chance of a successful IVF/PGD procedure is potentially decreased. It is noteworthy that BRCA2 deficiency in mice and zebrafish was also shown to result in impaired gametogenesis (Sharan et al., 2004; Rodriguez-Marí et al., 2011).

Different ranges of trinucleotide (CGG) repeats within the 5′-untranslated region of the fragile X mental retardation gene (FMR1),
which codes for FMR1 protein (FMRP), are associated with different phenotypes in women: over 200 repeats (full mutation) are associated with mental retardation, which is usually mild compared with men with a full mutation; 55–199 repeats (pre-mutation) are associated with premature ovarian failure (Sherman, 2000); alleles with repeats <28 and in the 34–55 range have also been associated with prematurely diminished ovarian reserve (Gleicher et al., 2009; Gleicher et al., 2010). The phenotypes observed correlate with the expression levels of FMRP, i.e. FMR1 translation has a peak when CGGn is in the normal range (around 30) and it decreases when the repeat length is smaller or larger (Chen et al., 2003).

A recent study has reported that the primary ovarian insufficiency (POI) among BRCA1/2-female mutation carriers is in fact due to a low FMR1 sub-genotype (CGG n < 26) (Weghofer et al., 2012). The authors also suggested that low sub-genotypes of the FMR1 gene might be important to rescue BRCA1/2+/- embryos, which would otherwise be embryonically lethal. Biallelic inactivation of BRCA1 is thought to be embryonically lethal, as observed in mice (Howlett et al., 2002). Additionally, no individuals with homozygous BRCA1 mutations were reported. Not even among the Ashkenazi Jewish population, who have a very high incidence of two specific BRCA1 and one BRCA2 mutation, BRCA2 compound heterozygotes are rare and these individuals are affected with Fanconi anaemia, type D1 (Hakem et al., 1996). It was observed that the embryonic lethality of BRCA1 -/- mice could be rescued by deletion of components of the DNA damage repair (DDR) pathway, such as p53, ATM, Chek2 and 53BP1 (Xu et al., 2001; Cao et al., 2006, 2009). We therefore found the FMR1 rescue hypothesis for the presumed embryonic lethality of human BRCA1/2+/- mutation carriers very interesting. Testing additional BRCA1/2 carriers, including males, who carry only one FMR1 allele, would allow us to confirm this hypothesis. Therefore, we aimed at testing the FMR1 sub-genotypes among BRCA1/2-mutation carriers in our centre.

Materials and Methods

Study population

In total, we tested the FMR1 sub-genotypes in 60 unrelated females (30 BRCA1- and 30 BRCA2-mutation carriers) and 29 unrelated males (14 BRCA1- and 15 BRCA2-mutation carriers) and 375 female controls. The female controls were relatives of Fragile-X syndrome patients and had been tested for FMR1 repeat size for diagnostic purposes but were anonymized for this study. After removing samples with at least one FMR1 allele containing CGGn >55, as these are pre-mutations, we had normal genotypes from 325 female controls.

DNA samples from BRCA1/2 mutation carriers were anonymized after selection and could not be traced back to the patient after genotyping and analysis, in agreement with the informed consent of these patients.

Genotyping

Assessment of the sub-genotypes was performed by PCR amplification using previously described primers (Monet et al., 1996). Size fragment analysis was performed by capillary electrophoresis in an ABI PRISM 3730 automatic sequencer (Applied Biosystems). Since this methodology only detects alleles up to a maximum of ~70 repeats, confirmation of FMR1 alleles with equal length was performed with the AmplideX™ FMR1 PCR Kit (CE IVD), allowing the exclusion of expansions of the FMR1 repeats. Genotypes and sub-genotypes are defined as previously reported by Gleicher et al. (2010). Briefly, a normal genotype refers to both alleles in the normal range (26 ≤ CGGn ≤ 34), homozygous refers to low–low, high–high or low–high sub-genotypes (i.e. both alleles outside the normal range), whereas heterozygous refers to normal–low or normal–high sub-genotypes (i.e. one allele in the normal range). Comparison of the sub-genotype distribution was performed using the χ² statistical test and 5% significance level.

Results

Our results for the female population are shown in Fig. 1 and the sub-genotype distribution from our and previously published female populations (Weghofer et al., 2012) are depicted in Fig. 2. Briefly, 45% of the female BRCA1/2-mutation carriers had two normal alleles (26 ≤ CGGn ≤ 34), 50% were heterozygous [20% had a normal and a high allele (n > 34), 30% had a normal and a low allele (n ≤ 26)] and the remaining 5% were homozygous (2% had high/low alleles and 3% were low/low, no high/high genotypes were found). There were no significant differences observed between the BRCA1 and BRCA2 populations (P = 0.333). The distribution of the FMR1 sub-genotypes among female BRCA1/2-mutation carriers resembles that of the controls in
our study and that from the control population used by Weghofer et al., whereas it is significantly different from the previously reported BRCA1/2 female population used by Weghofer et al. (P-value < 0.0001). Among male carriers, the majority had the normal genotype (26 ≤ CGGn ≤ 34), only eight had the low genotype (20%) and three male carriers had a high genotype. These allelic distribution frequencies are not different from those of both BRCA1/2 and control female populations analysed (Fig. 3).

**Discussion**

Weghofer et al. recently reported that low FMR1 sub-genotype (CGG n < 26) explained the POI among BRCA1/2-female mutation carriers. The association found by the authors was so strong that they have also suggested that low sub-genotypes of the FMR1 gene might be important to rescue human BRCA1/2+/- embryos, in which the mutation would otherwise be embryonically lethal (Weghofer et al., 2012). The results...
published by Weghofer et al. would have great implications for women undergoing an IVF procedure for fertility preservation or subsequent embryo selection by PGD. We aimed at testing their hypothesis in our BRCA1/2 population. We have also included males, to confirm the embryo rescue hypothesis, since these carry only one FMR1 allele on the X chromosome.

Our findings contrast with those from previously reported by Weghofer et al. An explanation for this discrepancy could be that Weghofer et al. used a control population from a different geographic region to where their BRCA1/2-mutation carriers originated (USA versus Austria, respectively). However, to test this hypothesis, Austrian women without BRCA1/2 mutations would have to be genotyped. Furthermore, Weghofer et al. suggested that in human embryos the BRCA1/2+/- mutation may be embryonically lethal. This was based on their results on the strong selection of FMR1 genotypes, since almost all subjects in their BRCA1/2 population had at least one low allele and only few normal/normal genotypes were observed. However, besides their work there is no evidence of embryonic lethality of human BRCA1/2+/- genotypes. Rescue of embryonic lethality of BRCA1-/- mice embryos was observed in the presence of a deletion of components of the DDR pathway, such as p53, ATM, Chk2 and 53BP1 (Xu et al., 2001; Cao et al., 2006, 2009). Although it cannot be excluded, Weghofer et al. did not provide experimental evidence that FMR1 is part of the DDR pathway.

In fact, POI in BRCA1/2-mutation carriers is most likely due to impairment of the BRCA1-related DNA damage repair mechanism itself, which causes an accumulation of double-strand breaks in oocytes leading to increased apoptosis (Titus et al., 2013). The results of this recent study suggest that ovarian reserve is affected in all BRCA1 carriers independent of their FMR1 genotypes. Nevertheless, to improve future association studies on this subject, parameters reflecting ovarian reserve, such as the level of anti-Müllerian hormone in the serum of age-matched groups, should be included. Also, larger populations should be studied to allow for separate analysis of BRCA1 versus BRCA2 carriers. To date, POI has not been observed among BRCA2 women, despite the fact that BRCA2 deficiency leads to impaired gametogenesis in animal models. In summary, if rescue of human embryos with BRCA1/2 mutations exists, it does not seem likely that it is due to the presence of low FMR1 sub-genotypes since we did not observe a selection towards low alleles in our BRCA1/2-mutation carrier population. Moreover, low FMR1 sub-genotypes do not appear to be the major mediators in POI observed in BRCA1-mutation carriers, but rather the effect of DDR pathway mutations as recently shown.

Authors’ roles
R.D.B. conceived the study, analysed and interpreted the data and drafted the manuscript. K.R. participated in the collection of data and revised the manuscript. D.T. collected data and revised the manuscript. M.J.B. participated in study conception and design of the study and revised the manuscript. All the authors approved the version to be published.

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Conflict of interest
None declared.

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