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SHORT REPORT

The *FMR1* CGG repeat test is not a candidate prescreening tool for identifying women with a high probability of being carriers of *BRCA* mutations

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The identification of women with a high probability of being carriers of pathogenic *BRCA* mutation is not straightforward and a major improvement would be the availability of markers of mutations that could be directly evaluated in individuals asking for genetic testing. The *FMR1* gene testing was recently proposed as a candidate prescreening tool because an association between *BRCA* pathogenic mutations and *FMR1* genotypes with 'low alleles' (CGG repeat number <26) was observed. To confirm this hypothesis, we evaluated the distribution of *FMR1* alleles and genotypes between *BRCA* mutation carriers and non-carriers in a cohort of 147 Italian women, free of cancer or affected by breast and/or ovarian cancer, who were tested for the presence of *BRCA* mutation in a clinical setting. The distribution of *FMR1* CGG repeat numbers in the two groups was similar (lower allele median/mean were 30/27.4 and 30/27.9, respectively; Mann–Whitney test $P=0.997$) and no difference in the *FMR1* genotype distribution was present ($\chi^2=0.503$, d.f. $=2$, $P=0.78$). This result is in contrast with literature data and suggests that *FMR1* genetic testing is not a candidate *BRCA* prescreening tool.
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INTRODUCTION

The decision to offer testing for *BRCA* mutations is challenging in many women in whom an inherited predisposition to breast/ovarian cancer is suspected, because of the associated costs, but also for the difficult interpretation of test results when genetic variants of uncertain significance are detected. Although several tools have been developed to help in this decision, their performance is unsatisfactory.[1]

A major improvement would be the availability of markers of pathogenic *BRCA* mutations that could be directly evaluated in individuals candidate to (or asking for) genetic testing.

A low ovarian response rate was observed in *BRCA1* mutation-positive women with breast cancer undergoing *in vitro* fertilization, suggesting an association between *BRCA1* mutation and occult primary ovarian insufficiency.[2] Impairment of functional ovarian reserve is associated with another genetic trait, the polymorphic CGG repeat of the Fragile X Mental Retardation 1 (*FMR1*) gene.[3] Female *FMR1* premutation carriers (55–200 CGG repeats) are at increased risk of primary ovarian insufficiency (FXPOI). However, an increased risk of ovarian insufficiency was also reported in women with CGG repeat numbers still within the conventional normal range (<55 CGG repeats) but lower (<26 CGG repeats) or higher (35–55 CGG repeats) of the range of CGG repeats associated with normal folliculogenesis (26–34 CGG repeats).[4–6]

Recently, Weghofer *et al*[7] have investigated whether *BRCA* mutations and *FMR1* ovarian genotypes are interdependent: in Austrian women carrying a *BRCA* mutation they observed almost exclusively the presence of genotypes containing at least one *FMR1* allele with a CGG repeat number below 26 (so called 'low *FMR1* genotypes').[7] To explain this unexpected finding, they speculated that *BRCA* mutations could be embryo-lethal unless rescued by genotypes containing low *FMR1* alleles and that the risk of prematurely diminished ovarian reserve reported as being associated to *BRCA1* mutations could be *FMR1* mediated.

If their findings are confirmed, *FMR1* testing could be considered as a potential prescreening tool since *BRCA* mutations carriers could be expected, almost entirely, amongst the 25% of women of the general population carrying low *FMR1* alleles.[8]

The present study was aimed at comparing the distribution of *FMR1* genotypes between *BRCA* mutation carriers and non-carriers in a cohort of Italian women, free of cancer or affected by breast and/or ovarian cancer, who were tested for the presence of *BRCA* mutation in a clinical setting because of their personal and/or familial cancer history.

MATERIALS AND METHODS

The study was approved by the local Ethical Committee.

Eligible to the study were unrelated Italian women, with or without breast cancer, who were enrolled at our center from January 2006 to April 2012 in a prospective study on familial breast cancer risk (Familial Breast Cancer Risk and Mutagen Sensitivity, AIRC Study), and had been tested for *BRCA* mutations for suspected Hereditary Breast Ovarian Cancer (HBOC) syndrome.

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To enrich the study sample of *BRCA* mutation carriers, unrelated women identified as *BRCA* mutation carriers at any time since our cancer genetics laboratory offered this type of testing (year 2004) were also selected. Eligible cases should have given their consent to the use of genetic information and DNA for research purposes at the time of genetic testing.

Results of *BRCA* genetic testing were derived from clinical records. Until May 2010, BRCA1/BRCA2 mutation screening was performed by denaturation high-performance liquid chromatography (dHPLC) followed by the direct DNA sequence analysis of fragments with altered dHPLC profiles. Subsequently, DNA direct sequencing was utilized as mutation screening method. Large deletions and duplications were excluded by multiple ligation probe amplification (MLPA) analysis using a commercial kit (MRC-Holland, Amsterdam, The Netherlands). *BRCA* variant classification followed international rules.[9] In particular, variants were classified as pathogenic if (i) they introduced a premature stop codon (frameshift, nonsense), (ii) they affected the highly conserved splice site positions AG/GT or (iii) they were missense variants whose clinical significance had already been demonstrated.

BRCA1 and *BRCA2* pathogenic variants are listed in Supplementary Table 1 and they have been submitted to the Breast Cancer Information Core database (http://research.nhgri.nih.gov/bic/).

The CGG repeat number of *FMR1* alleles was determined by capillary gel electrophoresis of fluorescent-labeled DNA fragments amplified using primers F (labeled with FAM fluorochrome) and C described by Fu *et al.*[10] The reactions were performed with the GC Rich PCR System (Roche Diagnostics, Basel, Switzerland); the fragments were separated on an automatic sequencer 3130xl Genetic Analyzer (Life Technologies, Foster City, CA, USA) using a 36-cm capillary, the POP7 polymer, and the Genescan ROX-500 (Life Technologies) as internal standard markers. Data were elaborated using Genescan v. 3.2.

For the purpose of comparison with Weghofer *et al*,[7] *FMR1* genotypes were defined according to the recently reported ovarian genotypes and sub-genotypes classification by Gleicher *et al.*[6] Based on a normal range of 26–34 repeats (median 30), *FMR1* alleles containing less than 26 CGG repeats were defined as 'low alleles' and *FMR1* alleles containing more than 34 CGG repeats as 'high alleles'. Genotypes were classified as *norm* (normal) if both alleles were in the normal range ($CGG_{n=26–34}$), *het* (heterozygous) if one allele was outside the normal range and *hom* (homozygous) if both alleles were outside normal range. *Het* individuals were subdivided into sub-genotypes depending whether abnormal alleles were above (*high*) or below (*low*) normal range.

The distribution of the CGG repeat number in women with and without *BRCA* mutation was compared by means of the Mann–Whitney test. The χ^2-test was used to compare the frequency of genotypes in the two groups.

RESULTS

In total, 147 unrelated Italian women were included in the study: 128 women consecutively enrolled in the prospective familial breast cancer AIRC study and additional 19 women identified as *BRCA* mutation carriers from year 2004.

Thirty-seven women (25.2%) were free of cancer, while the remaining women had a history of breast cancer ($n=101$, 68.7%), ovarian cancer ($n=3$, 2%) or both breast and ovarian cancers ($n=6$, 4.1%).

In the AIRC group, a pathogenic *BRCA* variant was found in 24 of 128 cases (18.75%). In the remaining 104 cases, the reported methods of *BRCA* screening varied with time (dHPLC in 58 cases, 55.8%, and direct DNA sequencing in 46 cases, 44.2%) and large deletions and duplications were excluded by MLPA analysis in 64 cases (61.5%).

Overall, 43 of 147 cases (29.25%) carried a mutation in a *BRCA* gene (23 in *BRCA1* and 20 in *BRCA2)*: 24 women from the AIRC study and 19 known mutation carriers.

The distribution of *FMR1* CGG repeat numbers in women with and without *BRCA* mutations is shown in Figure 1 and was similar in the two groups of women: the median/mean(SE) CGG repeat numbers in the lower allele were 30/27.4(0.71) and 30/27.9(0.37) in *BRCA* carriers and non-carriers, respectively (Mann–Whitney test $P=0.997$). No difference in the *FMR1* genotype distribution is present among the two groups (Figure 2): among *BRCA* mutation carriers, 22 (51.2%) were *norm,* 18 (41.9%) were *het* and 3 (6.9%) were *hom*, while in non-carriers 53 (51%) were *norm*, 40 (38.5%) were *het* and 11 (10.5%) were *hom* ($\chi^2=0.503$, d.f. $=2$, $P=0$ 0.78).

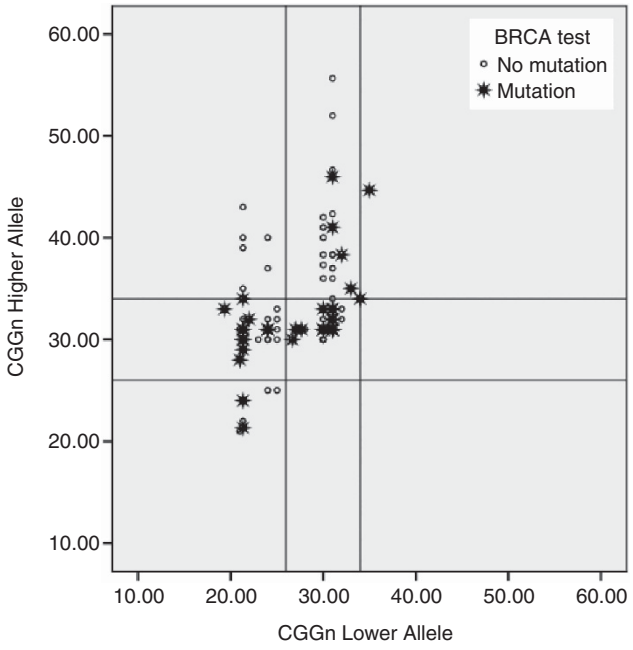


Figure 1 Distribution of both FMR1 alleles in women with *BRCA* mutations (black stars) and in women with *BRCA* test inconclusive (white dots) in form of scatter plot. The *norm* genotype ($CGG_{n=26–34}$) is defined by horizontal and vertical parallel lines.

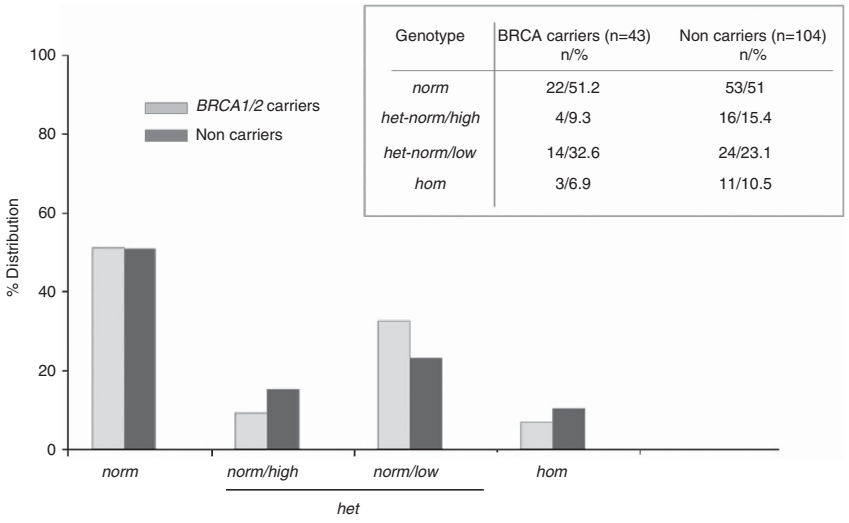
Similarly, no difference in the distribution of *FMR1* genotypes was observed when women with or without cancer were compared (data not shown).

DISCUSSION

In the present study we evaluated the association between *FMR1* CGG repeat genotypes and *BRCA* mutations in 147 unrelated Italian women who have undergone *BRCA* genetic testing in a clinical setting because of their personal and/or familial history of breast/ovarian cancer. No difference in the *FMR1* CGG repeat numbers or in the distribution of *FMR1* genotypes and sub-genotypes was observed between *BRCA*-positive and *BRCA*-negative groups (Figures 1 and 2).

This result is in contrast with the finding reported in Austrian *BRCA* mutation carriers by Weghofer *et al.*[7] In their study, these authors showed a significantly different distribution of *FMR1* genotypes in *BRCA* women compared with a control group of women (patients with infertility problems). In particular, the *FMR1* alleles distribution in the control population was comparable to the one reported in the general population (and in the present study), while almost all (93 of 99) Austrian *BRCA* mutation carriers presented with at least one low *FMR1* allele (<26 CGG repeats).

The discordant results of the two studies are difficult to explain, even though differences in the study design do exist. In our study,



Genotype	BRCA carriers (n=43) n/%	Non carriers (n=104) n/%
norm	22/51.2	53/51
het-norm/high	4/9.3	16/15.4
het-norm/low	14/32.6	24/23.1
hom	3/6.9	11/10.5

Figure 2 *FMR1* genotypes and sub-genotypes in women with (gray bars) and without (black bars) *BRCA* mutations.

only unrelated women were included while it is not clear if this is the case also in the study by Weghofer *et al*,[7] particularly for the *BRCA*-positive group (64 different *BRCA* mutations are reported for 99 index cases). Another difference between the two studies is the source of the control group, as in our study the control population is represented by a prospective cohort of women who performed *BRCA* genetic testing for clinical purposes while the control population of the Weghofer *et al*[7] study was not tested for *BRCA* mutation. However, this fact does not explain the difference in results of the two studies, which was due entirely to the difference in the two groups of *BRCA* carriers.

Alternatively, this difference might not be due to study bias but to a true difference between Italian and Austrian *BRCA* mutation carriers. This hypothesis, though unlikely, cannot be ruled out because at present little is known about the nature and distribution among different populations of *BRCA*-associated functional interacting factors.

A limitation of our study is the sample size. However, the frequency of normal genotypes in BRCA carriers (22/43, 51%) is similar to that observed in non-carriers, in sharp contrast with the almost complete absence of all constitutional genotypes except for sub-genotypes with low FMR1 alleles detected in the Austrian BRCA mutation carriers, and cannot be attributed to chance alone.

Certainly, these contradictory data require further investigation in other cohorts of women undergone BRCA genetic testing, including series of cases with different ethnic background.

In conclusion, our findings suggest that, so far, FMR1 testing cannot be considered as a prescreening tool to identify individuals with a higher probability of being carriers of pathogenic BRCA mutations.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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