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# Association of BRCA1/2 mutations with FMR1 genotypes: Effects on menarcheal and menopausal age

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ABSTRACT

*Objective:* Female BRCA (breast cancer gene)-1 and BRCA-2 mutations are significantly associated with risk of developing breast and ovarian cancers, in turn, associated with female infertility. BRCA-1 mutations have also been associated with occult primary ovarian insufficiency (OPOI), as have different mutations of the FMR1 gene. We, therefore, hypothesized that FMR1 genotypes may be associated with menarcheal and menopausal ages of BRCA mutation carriers.
*Patients:* We compared the FMR1 genotype and sub-genotype distribution in 99 BRCA1/2 positive women and in 182 healthy women without a known history of familial breast and ovarian cancer and searched for associations with age at menarche and menopause. *T*-test was used to assess differences in menarcheal and menopause ages, with times of menarche and menopause as continuous variables.
*Results:* Women with BRCA1/2 mutations showed significantly different FMR1 genotype and sub-genotype distributions when compared with the control group ($p < 0.001$). This result remained stable in a sub-group analysis of Caucasian BRCA1/2 carriers and healthy controls ($p < 0.001$). In addition, BRCA1/2 carriers indicated a trend toward shorter reproductive lifespan ($p = 0.18$).
*Conclusions:* Our data confirm the previously reported highly skewed distribution of FMR1 genotypes and sub-genotypes toward a high preponderance of low FMR1 alleles in BRCA1/2 carriers. We could demonstrate that BRCA-1 mutations are associated with an earlier onset of menopause compared to BRCA-2 carriers, although the distribution of the het-norm/low genotype is similar in both groups. Our findings suggest that there may be other factors beside the genotype that has an influence on menarche and especially menopause age in BRCA mutation carriers.


## 1. Introduction

Female BRCA mutation carriers experience a lifetime breast cancer risk of up to 80 percent [1]. Since breast cancer in those women is typically diagnosed at a very young age [2], ovarian tissue banking and in vitro fertilization have been proposed to preserve fertility during cancer treatment [3,4].

Interestingly, BRCA mutation carriers who undergo fertility preservation treatments such as controlled ovarian hyperstimulation have recently been reported to experience a poorer follicular response than infertility patients undergoing the same procedure [5].

Since abnormal ovarian function testing and poor response to gonadotrophins during assisted reproduction are considered surrogate parameters of prematurely diminished ovarian reserve [5,6] these findings may point toward alterations in ovarian function in young female cancer patients with BRCA mutations.

Although a variety of environmental factors, such as radiation and ovarian surgery, are known to prematurely deteriorate ovarian function, the duration of a woman's reproductive lifespan as well as ovarian aging patterns appears to be mainly genetically pre-determined [7–10].

FMR1 (fragile X mental retardation 1) gene codes for a protein named fragile X mental retardation protein. This protein is essential for cognitive development and also female reproductive function. Mutations of this gene can lead to fragile X syndrome, learning delay, autism, Parkinson's disease as well as to premature ovarian failure [11,12].

Among a number of candidate genes, the association between premature ovarian insufficiency and the X-chromosome's FMR1 gene serves as a prime example for a genetic implication of human reproduction [13,14]. Beside the well-established association between increased CGG repeat numbers on the FMR1 gene and premature ovarian failure (POF), CGG repeat ranges previously considered normal have recently been associated with

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prematurely diminished ovarian reserve. These ovarian genotypes and sub-genotypes of the FMR1 gene may, indeed, determine different ovarian aging patterns, some being at risk for premature ovarian insufficiency [15,16].

Particularly, women with the het-norm/low FMR1 sub-genotype, who demonstrate one allele below the repeat range of 26–34 CGG repeats, have previously been suggested at risk for prematurely diminishing ovarian function [17]. In an attempt to evaluate whether different distributions of FMR1 genotypes and sub-genotypes are apparent in BRCA mutation carriers and may, thus, explain previous findings on occult primary ovarian insufficiency in women with BRCA mutations, we have previously compared FMR1 genotype and sub-genotype distributions between BRCA carriers and infertile women [18].

Since women with premature ovarian insufficiency are, however, disproportionately highly represented in infertility cohorts, the present study was initiated to compare FMR1 distribution patterns between BRCA women and healthy controls with normal ovarian function testing.

## 2. Materials and methods

### 2.1. Patients

All 281 participating women had signed informed consent forms for medical and genetic testing for medical and research purposes. Institutional Review Boards (IRB) approvals were obtained for the respective patient populations by the IRB of the Medical University of Vienna and by the IRB of The Center for Human Reproduction.

To compare FMR1 genotype and sub-genotype distributions between BRCA mutation carriers and healthy controls, the present study involved molecular testing of the FMR1 gene and retrospective chart review in 281 women. Ninety-nine Austrian female BRCA mutations carriers (50 BRCA-1 and 49 BRCA-2 carriers) represented the study group and 182 healthy American women with uneventful personal and medical histories served as controls.

All BRCA patients participated in a screening program for hereditary breast and ovarian cancer at the Department of Obstetrics & Gynecology at the Medical University of Vienna and underwent FMR1 testing.

Healthy controls had volunteered as oocyte donation candidates at The Center for Human Reproduction in New York, NY, USA, and had undergone medical and genetic testing that included FMR1 genotype analysis as parts of the screening process prior to oocyte donation.

### 2.2. Molecular analyses

Until December 2008, BRCA1/2 analyses were performed using denaturation high performance liquid chromatography, as previously reported [19]. After 2008, DNA sequencing, with use of chain-terminating inhibitors was utilized [20].

FMR1 testing in both institutes was performed as previously reported [21,22]. *FMR1* genotypes were assigned based on a previously reported normal range of 26–34 (median 30) CGG repeats according to Gauss' distribution [23]: Normal was defined by both alleles within the range of 26–34 CGG repeats, while abnormal was defined by the presence of one or both alleles above or below the normal range of 26–34 CGG repeats [24].

### 2.3. Statistical analysis

Chi-square analysis was performed to explore the relationship between FMR1 genotypes and sub-genotypes and BRCA group membership. *T*-tests were used to assess differences between ages at menarche and menopause among groups. Results are given as *p*-values. A $p$-value < 0.05 was considered statistically significant. All statistical calculations were performed utilizing SPSS 18.0 (Chicago, IL).

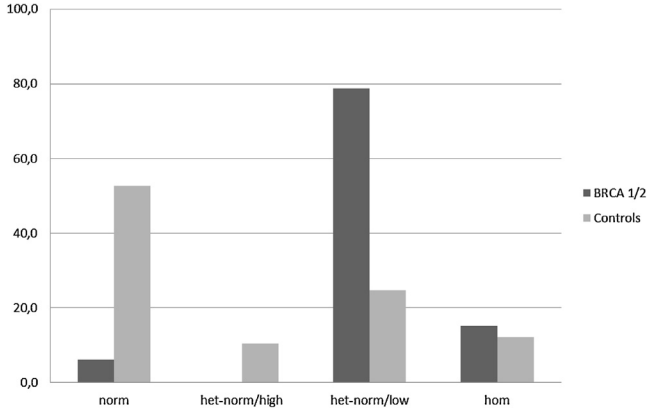


**Fig. 1.** Definition and distribution of FMR1 genotypes and sub-genotypes (%) in BRCA1/2 mutation carriers (dark gray) compared to the healthy control group (light gray).

| Group membership | Allele 1 CGG range between | Allele 2 CGG range between |
|---|---|---|
| Norm | 26 and 34 | 26 and 34 |
| Het-norm/high | 26 and 34 | ≥35 |
| | ≥35 | 26 and 34 |
| Het-norm/low | 26 and 34 | ≤25 |
| | ≤25 | 26 and 34 |
| Hom | ≥35 | ≥35 |
| | ≤25 | ≤25 |

| *n* (%) | BRCA1/2 mutation carriers | Healthy control group |
|---|---|---|
| Norm | 6(6.1) | 96(52.7) |
| Het-norm/high | 0(0) | 19(10.4) |
| Het-norm/low | 78(78.8) | 45(24.7) |
| Hom | 15(15.2) | 22(12.1) |

## 3. Results

Distributions of FMR1 genotypes and sub-genotypes in BRCA patients and controls are presented in Fig. 1. Among healthy women, 52.7% showed normal FMR1 genotypes, 35.1% tested heterozygous (24.7% het-norm/low and 10.4% het-norm/high, respectively) and 12.1% presented with homozygous FMR1 genotypes. This distribution of $CGG_n$ follows that observed in infertility patients and in general populations [4,23,25].

BRCA1/2-positive women, in contrast, presented with significantly different FMR1 genotype and sub-genotype distributions ($\chi^2(3,\ n=281)=92.11,\ p\leq 0.001$): Only 6.1% of BRCA positive women showed normal FMR1 genotypes. The majority of all BRCA women, i.e. 78.8 percent, showed heterozygous genotypes (74.0% in BRCA-1 and 83.7% in BRCA-2 patients, respectively). However, heterozygotes presented exclusively with het-norm/low genotypes, no het-norm/high sub-types were detected. Homozygote genotypes were exhibited in 15.2% of all BRCA positive women.

To account for ethnicity as a potential confounding factor of FMR1 genotype distribution in US and Austrian women, a subgroup analysis that included Caucasian women only was performed, which included 99 BRCA positive women and 123 controls. A significant relationship was observed between FMR1 genotypes and BRCA group membership ($\chi^2$ $(3,\ n=222)=76.97,\ p\leq 0.001$). In the study group, 78.8% Caucasian women showed the het-norm/low sub-genotype, while 30.1% of het-norm/low FMR1 sub-genotypes were observed among Caucasian controls. In Caucasian BRCA