NEWS AND COMMENTARY

BRCA and FMR1

Do *BRCA1/2* mutations and *low FMR1* alleles interact or not?

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Ricci *et al*¹ report in this issue that, contrary to prior suggestions,² *FMR1* mutations may not be predictive of *BRCA* mutations status. Collaborating with Austrian colleagues, we in 2011/2012, almost accidentally, stumbled over unexpected results when looking at CGG repeats (CGGn) on the *FMR1* gene in carriers of *BRCA* mutations (*BRCA1* and *BRCA2*).² The idea for this study came from reports that selected *FMR1*^{2,3} and *BRCA1*⁴ mutations, independently, appeared associated with premature ovarian senescence. Austrian colleagues, who had access to a range of *BRCA1/2* mutation samples (64 different mutations) from a local Austrian population, and ability to utilize a well-established *FMR1* assay, graciously offered their cooperation.²

BRCA1/2 and *FMR1* results from investigated patients were, independently, transmitted from two University centers in Graz and Vienna to our center in New York, where associations were determined. Mutations of *FMR1* were defined based on a newly defined 'normal' CGGn of 26–34.³ Based on this range, patients are defined as normal (*norm*, with both alleles in range), as heterozygous (*het*, with one allele in and one outside range) and as homozygous (*hom*, with both alleles outside range). *Het* and *hom* patients are then further sub-categorized as *low* (CGGn < 26) or *high* (CGGn > 34).

Utilizing these newly defined mutations, we reported significant associations with

ovarian aging patterns,^{2,3} ethnicities/races,^{5,6} evidence for immune system activation² and, most recently, methylation patterns.⁷ Most striking variations were seen among *het* mutations, with *low* alleles associated with the most severe adverse outcomes, like lowered pregnancy chances with *in vitro* fertilization (IVF),^{2,6} increased risk toward immune system activation^{2,6} and abnormal methylation.⁷

In different female populations *norm* represented uniformly approximately 55–60% of women, *het* 40–45% and *hom* approximately 6–8%, a distribution pattern also confirmed in Austrian non-*BRCA* carrier women.² Austrian *BRCA1/2* mutation carriers, however, differed to surprising degrees: they demonstrated almost complete absence of all constitutional *FMR1* genotypes except for those with *low* (CGGn < 26) alleles.²

In their here presented report Ricci *et al*¹ repeated this study, though with greatly differing results. Largest differences were seen in *norm* and *het-norm/low* women: while Austrian *BRCA1/2* mutation carriers, comparatively, demonstrated significantly reduced numbers of *norm FMR1* genotypes (6.1 vs 58.0%) and significantly increased numbers of *het-norm/low* (78.8 vs 20.5%), Italian *BRCA1/2* carriers demonstrated practically identical numbers for *norm FMR1* genotypes as non-carriers (51.2 vs 51.0%), although still somewhat elevated numbers for *het-norm/low* (32.6 vs 23.1%). *Het-norm/high* and *hom* patients (combined for all sub-genotypes) in both studies represented small numbers, and, therefore, appear insignificant for this discussion.

Observed differences between the data reported by Ricci *et al*¹ and ours,² therefore, raise three important questions; the first one being why are there such differences

between studies performed in neighboring countries?

Explanations can, most likely, be found in two significant differences between the two studies: both studies are small but the Italian study is even smaller (43 vs 99 patients), increasing risks for patient selection biases. At 51.0%, for example, the *norm FMR1* population appears significantly underrepresented in comparison to average populations in Austria² and the United States.^{2,8} Likely more important is, however, that none of the 32 *BRCA1/2* mutations in the Italian study was also represented among 64 different mutations observed in Austrian patients. In other words, Austrian and Italian women represented completely different *BRCA1/2* mutations.

This may surprise, especially in neighboring countries. Austria and Italy, however, are known to demonstrate highly varying *BRCA* mutations (Table 1),⁹ and even in very small regions within Europe, and in relatively small populations considerable variations in *BRCA* mutations have been reported.^{9,10}

If one assumes that differences in *BRCA* mutations in both studies are responsible for variations in the distribution of observed *FMR1* mutation, the next question to be resolved is why different *BRCA* mutations may affect *FMR1* mutation distribution differently?

Observing in Austrian *BRCA* mutation carriers, with relatively few exceptions, only *low FMR1* alleles, we concluded that *BRCA* mutations, almost universally, had to be embryo-lethal, unless rescued by a *low FMR1* allele.² Ricci *et al*¹ now, rightly, questions our conclusions, and suggest that *low FMR1* alleles, therefore, cannot be used as a surrogate for *BRCA1/2* screening, as we had suggested. They may be correct if the small study size of our data set resulted in an unfortunate statistical coincidence. Another possible explanation for observed discrepancy in findings, however, is that not all but only selected *BRCA1/2* mutations are embryo-lethal. Interesting to note in such a context is that even Ricci's Italian *BRCA* mutation carriers, comparatively, demonstrate nominally more *het-low FMR1* alleles than their own non-carriers (32.6 vs 23.1%) and than reported by us in general populations.^{2,8}

Rodent models of *BRCA* support potential embryo lethality of the *BRCA* gene, though degrees of lethality vary between models,² suggesting that embryo lethality, indeed, may vary between *BRCA* mutations.

We hypothesized, based on noted Austrian patient data, that rescue of *BRCA* mutation-carrying embryos by a *low FMR1* allele may

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Table 1 Most common *BRCA1/2* mutations in Austrian and Italian women^a

<i>BRCA1</i>		<i>BRCA2</i>	
HGVS	BIC	HGVS	BIC
<i>Austrian</i>			
c.181T>G	300T>G	c.8363G>A	8591G>A
c.5266dupC	5382insC	c.8754 + 1G>A	IVS21-1G>A
c.1687C>T	1806C>T	c.3860delA	4088delA
c.3016_3019del14	3135del14		
c.2676_2679del14	2795del14		
<i>Italian—Tuscany</i>			
c.3228_3229delAG	3347delAG		
c.3285del	3404delA		
c.1380dupA	1499insA		
c.5062_5064del3	518delGTT		
<i>Sardinia</i>			
		c.8537_8538delAG	8765delAG
<i>Calabria</i>			
c.4964_4982del19	5083del19		
<i>North-east</i>			
c.5062_5064delGTT	5181_5183delGTT		

^aAfter Janavičius R;⁹ True founder mutations are in *italic*; HGVS, Human Genetic Variation Society; BIC, Breast Cancer Information Core data-base; *BRCA1* numbered by GeneBank U14680 reference sequence; *BRCA2* numbered by GeneBank U43746 reference sequence.

explain the so-called 'BRCA paradox,' defined by antiproliferative effects of *BRCA* in embryonic tissue (ie, potential embryo lethality) and proliferative activity in malignancies.² We furthermore hypothesized that *low FMR1* alleles, in rescuing *BRCA* mutation carrying embryos, block antiproliferative effects of *BRCA1/2*. Surviving embryos, therefore, lack these antiproliferative characteristics of their *BRCA1/2* genes, resulting in surviving adults being exposed to proliferative effects and *BRCA1/2*-associated cancer risks.

The study of Ricci *et al*¹ allows for a mild adjustment of this hypothesis: since now not all *BRCA1/2* mutations appear equally embryo-lethal, not all *BRCA1/2* mutations require rescue from embryo lethality by a *low FMR1* allele. Only embryo-lethal *BRCA1/2* mutations will require such rescue. Only adults with potentially embryo-lethal *BRCA1/2* mutations, therefore, will experience the *low FMR1* allele-induced effects of blocked antiproliferative *BRCA1/2* mutations. A so expanded *BRCA/FMR1* hypothesis, therefore, suggests that primarily embryo-lethal *BRCA1/2* mutations are also oncogenic.

This modified hypothesis of potential *BRCA/FMR1* interactions, paradoxically, is

actually easier to confirm (or refute) than the original hypothesis: if correct, *low FMR1* alleles can be expected to be disproportionately more frequent in breast and ovarian cancer-stricken *BRCA1/2* mutation carriers in comparison to patients with the same cancers lacking *BRCA* mutations. We are, indeed, currently investigating this question in a North American patient population, and are looking forward to similar investigations from Ricci *et al*¹ and colleagues in other countries.

As proposed in our initial publication,² the interplay between *BRCA* and *FMR1* genes, as of this moment, remains a hypothesis in need of confirmation. Sometime in the future the paper by Ricci *et al*¹ may be viewed as a first step in its confirmation or repudiation. Either way, their work deserves appreciation ■

CONFLICT OF INTEREST

AW has no potential conflicts to report. NG and DHB are listed as co-owners of a number of already awarded US patents relating to beneficial therapeutic effects of androgen supplementation on female infertility. Pending patents relate to diagnostic claims that the *FMR1* gene can be utilized to assess

ovarian aging, and to predict fertility treatment chances in association with IVF. NG is a shareholder in Fertility Nutraceuticals, LLC, and owner of the Center for Human Reproduction (CHR). The CHR supported this study through salary support to NG and DHB. NG and DHB receive patent royalties from Fertility Nutraceuticals, LLC.

AUTHOR CONTRIBUTIONS

NG was invited to present this commentary and developed the concept for the manuscript. AW and DHB contributed to the analysis of the manuscript by Ricci *et al*,¹ writing and editing of the manuscript. All authors approved the final manuscript before submission.

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