Comparison of ovarian *FMR1* genotypes and sub-genotypes in oocyte donors and infertile women

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Abstract

Purpose We recently described ovarian genotypes and sub-genotypes of the *FMR1* gene with distinctly associated ovarian aging patterns, which in infertile women follow a typical X-linked distribution pattern. Whether normally fertile women, however, also demonstrate the same distribution, is unknown. **Methods** We, therefore, investigated ovarian *FMR1* genotype and sub-genotype distribution in 182 oocyte donor candidates in comparison to 339 infertile controls. As previously reported, genotype designation was made, based on a normal range of CGG $n=26-34$ (median 30), defining women as normal (*norm*), heterozygous (*het*) or homozygous (*hom*). *Het* and *hom* genotypes were further subdivided into

sub-genotypes, based on whether abnormal alleles were above (*high*) or below normal range (*low*).

Results Oocyte donors presented with 47.8% *norm*, 45.1% *het* and 7.1% *hom* genotypes, confirming a typical X-linked distribution pattern. They, however, still subtly differed from infertility patients, especially in *het* sub-genotypes.

Conclusion These findings validate recently newly described ovarian genotypes and sub-genotypes, reaffirming their relevance to female fertility/infertility.

Keywords Fragile X mental retardation 1 gene (*FMR1*) · Ovarian reserve · Genotypes · Sub-genotypes · Oocyte donors · Infertility

Capsule Egg donor candidates were found to have a similar, but not identical, distribution of ovarian *FMR1* genotypes and sub-genotypes, compatible with previously reported functions.

N.G. and D.H.B. are listed as co-inventors on a pending U.S. patent application, which claims diagnostic benefits in infertility from assessing *FMR1* genotypes and sub-genotypes.

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Introduction

We previously reported ovarian *FMR1* genotypes and sub-genotypes, which were based on statistical analyses of the gene's CGG triple nucleotide repeats (CGG_n) in exclusively infertile patients [1–3]. These newly defined genotypes and sub-genotypes have to be differentiated from traditional *FMR1* genotypes, historically defining primarily neuropsychiatric risks, including the fragile X syndrome [4].

Traditional *FMR1* genotypes include common (normal) at CGG_{n<44}, intermediate (or gray zone) at CGG_{n=45–54}, premutation range at CGG_{n=55–200} and full mutation (fragile X syndrome) at CGG_{n>200} [4]. In contrast, recently reported ovarian genotypes of *FMR1* are based on the recognition that CGG_n in female populations is distributed in typical X-linked fashion, defined by a normal range of CGG_{n=26–34} (median 30) [1–3]. This median has previously been reported as switching point between positive and negative message, and as point of peak translation for the gene [5].

Depending on CGG_n on both X chromosomes, a woman, thus, can be normal (*norm*) if both alleles are in normal range, heterozygous (*het*) if one allele is outside normal range or homozygous (*hom*) if both alleles are outside range.

Het and *hom* genotypes can be further sub-divided into sub-genotypes, depending on whether abnormal counts are above (*high*) or below (*low*) normal range. *Het* can, thus, be sub-divided into *het-norm/high* and *het-norm/low*, while *hom* can be sub-divided into *hom-high/high*, *hom-high/low* and *hom-low/low* [2, 3]. Prior studies in 339 infertile patients demonstrated that approximately half demonstrated the *norm* genotype, around 40% the *het* genotype, with slightly more showing *het-norm/low* than *het-norm/high*, and remaining women demonstrating the various *hom* sub-genotypes [2]. Genotype and sub-genotype distribution to a degree varies between races/ethnicities, with women of African descent demonstrating disproportionately *het-norm/low* sub-genotypes, while Asian women demonstrate excessive *het-norm/high* sub-genotypes in comparison to Caucasian women [3].

CGG triple nucleotide repeats in infertile women are, however, to some degree skewed since low as well as high repeat numbers, have been associated with increased risk towards prematurely diminished ovarian reserve, also given the acronym of premature ovarian aging (POA) [6]. Since POA patients represent a significant portion of infertility patients [7], their distribution of CGG repeats may somewhat differ from the distribution in normally fertile women.

Carefully screened oocyte donors, of course, should represent normally fertile women. Validation of distributions of *FMRI* genotypes and sub-genotypes in oocyte donors, therefore, would reaffirm in infertile women established criteria for definition of ovarian *FMRI* genotypes and sub-genotypes and, in addition, validate these newly described ovarian *FMRI* genotypes and sub-genotypes as physiologically relevant.

The purpose of this study was, therefore, a comparison of distribution patterns of *FMRI* genotypes and sub-genotypes in normal oocyte donors and in, previously reported, infertile women [2, 3].

Materials and methods

This study was performed at the Center for Human Reproduction in New York City. In the U.S., the law permits anonymous oocyte donation, and oocyte donors are allowed to receive reasonable remuneration for time and efforts. Our center, therefore, maintains a large pool of prescreened oocyte donors to allow recipients choice between donors, and a quick recipient/donor matching process.

In order to be included into the center's donor pool, candidates have to be under age 34 years (but usually are under age 30), and undergo a very detailed, multi-step screening process, which excludes candidates with medically relevant

personal, family or genetic histories. Screening steps include an extensive written questionnaire and two face-to-face staff interviews, the latter one with a physician. Less than five percent of original applicants reach a final medical testing stage. One, therefore, cannot completely rule out the possibility that this very detailed screening process in unrecognized ways affects *FMRI* genotype and sub-genotype distribution.

The candidates' medical screening includes ovarian reserve assessments by ultrasound and measurements of anti-Müllerian hormone (AMH). Candidates with abnormally low AMH levels are usually excluded, though, in exceptional cases, may be allowed to donate with a modified ovarian stimulation protocol.

Since 2008, screening of donor candidates involves evaluation of the *FMRI* gene to exclude carriers of traditional premutation range CGG triple repeats (CGG_{n=55–200}) and of full mutations (CGG_{n>200}) [4]. Donor candidates undergo *FMRI* testing as part of a medical testing round, representing the last stage of screening prior to admission into the center's oocyte donor pool.

The donors' young age and their detailed screening virtually guarantee that none of the candidates suffers from significant ovarian function abnormalities. They, therefore, with reasonable certainty should approximate a normally fertile, young population of women.

In this study CGG_n of both alleles were determined in 182 consecutive oocyte donor candidates and 339 previously reported infertility patients [2, 3]. Utilized commercial tests to assess CGG_n and distribution of *FMRI* genotypes and sub-genotypes in this infertile population were also reported before [2, 3]. Since oocyte donor candidates are chosen to reflect the center's patient pool, we expected them to be similar in race/ethnicity distribution. This was confirmed (Table 1).

Baseline characteristics of both groups were tabulated using means and standard deviations or percentages. Comparisons between groups were made by Chi-Square or Fisher's Exact

Table 1 Patient characteristics of both groups

	Infertile Patients (n=339)	Donors (n=182)
Age (years)	37.9±5.0	24.0±3.8 *
BMI (kg/m ²)	24.4±4.8	21.3±2.6 *
AMH (ng/mL)	1.4±1.7	4.4±2.8 *
FSH (mIU/mL)	11.0±6.4	6.5±3.2 *
Estradiol (pg/mL)	53.1±39.9	54.0±35.4
Race (n/%)		
African	45 (13.3)	28 (15.4)
Asian	61 (18.0)	45 (24.7)
Caucasian	221 (65.2)	98 (53.8)
Other	12 (3.5)	11 (6.0)

Values are in means±standard deviations unless otherwise indicated.
*=P≤0.001

Test. Statistical analyses were undertaken using the Statistical Package for the Social Sciences 17.0 (SPSS, Chicago, IL, USA).

Like other patients, donors sign at time of initial consultation an informed consent that allows for use of their medical records for research purposes, as long as content of the medical record remains confidential and their identity remains protected. Both conditions were met for this study, which, therefore, qualified under the center's Institutional Review Board (IRB) rules for expedited review. Donors, in addition, signed a specific *FMR1* testing consent. Such consents are required for all genetic tests performed at the center.

Donor candidates' ovarian *FMR1* genotypes and/or sub-genotypes are currently not considered in deciding on qualification for the center's donor pool, since *FMR1* testing is performed to detect traditional premutation and full mutation range genotypes [4]. The center's IRB, however, mandated that, based on results from prior studies [1–3, 6], donors be informed if mutations from the *norm* genotypes are observed.

Results

Table 1 summarizes basic patient characteristics for both study groups. As the table demonstrates, egg donors were significantly younger ($P<0.001$) and had a lower BMI ($P<0.001$). They also demonstrated significantly higher AMH ($P<0.001$) and lower FSH values ($P<0.001$).

The 182 consecutive oocyte donor candidates demonstrated the following genotype and sub-genotype distribution (Fig. 1): *norm* 87 (47.8%); *het* 82 (45.1%); *hom*, 13 (7.1%). *Het* sub-genotypes were equally divided (41, 22.5%, each) between *het-*

Fig. 1 Distribution of *FMR1* genotypes and sub-genotypes in oocyte donors. Here presented distribution data of 339 infertile patients have previously been reported [2, 3]. Including *hom* patients/donors, the distribution differed between patients and donors (Chi-Square, $P<0.0001$). Individual genotype and sub-genotype distributions, however, did not differ significantly, though differences in distribution of *het* sub-genotypes demonstrated a trend (One-tailed Fisher's Exact Test, $P=0.08$)

norm/high and *het-norm/low*. *Hom* sub-genotypes were too few in number to offer statistically valid percentages.

FMR1 genotypes and sub-genotypes for the 339 infertile women, as reported before [2, 3], were: *norm* in 183 (54.0%); *het* 156 (46.0%); and the group contained no *hom* patients. *Het* sub-genotypes were: *het-norm/high* 62 (18.3%); and *het-norm/low* 94 (27.7%).

Including *hom* patients, the infertile patient population differed significantly between infertility patients and donors [χ^2 (3, $N=521$)=27.46, $P<0.001$], though individual genotypes and sub-genotypes did not differ between the two groups. The difference in distribution of *het* sub-genotypes demonstrated a trend (One-tailed Fisher's Exact Test, $P=0.08$).

Discussion

As this study demonstrates, ovarian *FMR1* genotype and sub-genotype distribution in oocyte donor candidates reflects a typical X-linked pattern. By approximately half of all donors (47.8%) demonstrating *norm* genotypes, and *het* genotypes, equally distributed (22.5% each) between *het-norm/high* and *het-norm/low*, donors follow such an X-linked pattern even marginally closer than infertile women.

As Fig. 1 demonstrates, donor distribution of *FMR1* genotypes and sub-genotypes mildly varies from that of infertile women (including *hom*, $P<0.0001$). While individual genotype/sub-genotype distribution differences lack significance, they appear most pronounced amongst *het* sub-genotypes, though, even there, fail to reach statistical significance ($P=0.08$).

These findings should not surprise: Small difference between supposedly normally fertile donors and an infertility population can be expected as both, abnormally low and high CGG_n, has been associated with POA [6]. POA, in turn, is, of course, highly associated with infertility.

Abnormally low and high CGG_n represents *het* and *hom* sub-genotypes, respectively [2, 3]. By definition, infertile women, therefore, can be expected to demonstrate a higher prevalence of *het* and *hom* genotypes.

Infertile women presented with almost identical total *het* genotype prevalence as donors (46% vs. 45%). Marginal differences in distribution were only seen amongst *het* sub-genotypes, where *het-norm high* represented 18.3% in patients but 22.5% in donors, and *het-norm/low*, with 27.7% in patients and only 22.5% in donors ($P=0.08$).

The distribution of *het* sub-genotypes in infertile women has previously been reported [2, 3]. *Hom* sub-genotypes, because of their low prevalence, however, so far are not well defined in their respective distribution and functions. This study, unfortunately, also lacks adequate numbers of *hom* individuals, and, therefore, does not further contribute to knowledge about distribution of *hom* sub-genotypes of *FMR1*.

Almost complete absence of *hom* genotypes in patients (Fig. 1) may, however, offer a first hint as to the significance of the *hom* genotype in infertility. Its extremely low prevalence suggests two possible explanations: women with *hom* genotype either have normal fertility or, probably more likely, a fertility level too poor for presentation to a fertility center. They already at young ages may be so badly affected that, by more advanced age, they no longer are represented in typical infertile patient pools. Further studies of *hom* patients are, however, very obviously urgently needed.

Norm genotypes were marginally more frequent in patients than donors (54.0% vs. 47.8%), which has to be considered surprising. Though statistically insignificant, this finding warrants follow up to preclude unintended consequences from our current screening of donor candidates.

Considering that the *het-norm/low* sub-genotype is strongly associated with a reduction in IVF pregnancy chances [2, 3], the relatively high prevalence of *het-norm/low* among infertility patients should not surprise. An increased prevalence of *het-norm/low* is also observed in women of African descent, who also demonstrate diminished pregnancy chances in IVF [3].

Women with *het-norm/high* demonstrate better IVF pregnancy rates than those with *het-norm/low* *FMRI* sub-genotype but lower rates than women with *norm* genotype [2]. Especially in older women, *het-norm/high* is actually associated with comparatively increased oocyte yields [8], and, therefore, likely better pregnancy chances. To find more *het-norm/low* and less *het-norm/high* in infertile women, especially older women, therefore, does not surprise.

Differences in genotypes and sub-genotypes distribution of the *FMRI* gene may also be racial/ethnic: While Caucasian, African and Asian women, all, demonstrate approximately equal *norm* distributions in the low 50% range, they vary considerably in *het* sub-genotype distributions: Asian women, for example, demonstrate more *het-norm/high* (29.2%) than Caucasian (16.8%) and African patients (15.3%). In contrast, African women demonstrate the highest prevalence of *het-norm/low* (32.2%) and Asian the lowest (18.8%), with Caucasians occupying the middle ground (28.4%) [3].

Here presented data in oocyte donor candidates, thus, validate previously reported distributions in infertile women [2, 3]. Observed subtle differences between these two distinct patient populations are actually supportive of proposed physiological functions of ovarian *FMRI* genotypes and sub-genotypes. Because associated with specific ovarian aging

patterns [1, 2], varying in distribution amongst races/ethnicities [3], and predictive of autoimmune risk [2], they, apparently, guide important physiologic functions. Further exploration of these new ovarian genotypes of *FMRI*, therefore, appears indicated.

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Authors' roles

N.G. and D.H.B. contributed equally to study design, study conduct and preparation of the manuscript, with N.G. being primarily responsible for writing the manuscript and D.H.B. being primarily responsible for data analysis. A.W. contributed to study design and A.K. contributed to data analysis. All authors reviewed and approved the final manuscript.