REVIEW

The impact of *FMR1* gene mutations on human reproduction and development: a systematic review

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Received: 11 February 2016 / Accepted: 27 June 2016 / Published online: 18 July 2016
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Abstract

Purpose This is a comprehensive review of the literature in this field attempting to put the *FMR1* gene and its evaluation into context, both in general and for the reproductive health audience.

Methods Online database search of publications with systematic review of all papers relevant to ovarian reserve and assisted reproduction was done.

Results Relevant papers were identified and assessed, and an attempt was made to understand, rationalize and explain the divergent views in this field of study. Seminal and original illustrations were employed.

Conclusions *FMR1* is a highly conserved gene whose interpretation and effect on outcomes remains controversial in the reproductive health setting. Recent re-evaluations of the commonly accepted normal range have yielded interesting tools for possibly explaining unexpected outcomes in assisted reproduction. Fragile X investigations should perhaps become more routinely assessed in the reproductive health setting, particularly following a failed treatment cycle where oocyte quality is thought to be a contributing factor, or in the presence of a surprise finding of diminished ovarian reserve in a young patient.

Keywords Reproduction · Genetics · *FMR1* · Fragile X · Assisted reproductive technology · Testing

Capsule *FMR1* is a highly conserved gene whose interpretation and effect on outcomes remains controversial in the reproductive health setting.

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Introduction

Verkerk et al. discovered the fragile X mental retardation 1 gene (OMIM 309550, *FMR1* or FRAXA gene) in 1991, in individuals found to have a chromosomal instability at the 5'-UTR exon 1 on the long arm of the X chromosome (Xq27.3), incidentally, a location closely connected to autoimmunity [100]. Highly conserved, this gene is characterized by a CGG triplet repeat expansion [100]. Belonging to genetic disorders called microsatellites (about 30 % of our genome), CGG repeats in contrast are quite unstable but can have profound physiological effects. The *FMR1* triplet repeat mutation was the first such disorder originally identified in the human genome, with other nucleotide repeat conditions, often exceeding three nucleotides, discovered later [65]. The position of *FMR1* in a non-coding exon is in fact a typical trait shared by other nucleotide repeat disorders, even though the reason of such association remains mysterious. At present, more than 30 genetic conditions, frequently presenting with neurological impairment, share an altered number of repeated nucleotides as an underlying pathogenic mechanism. To date the *FMR1* gene mutation is undoubtedly the most investigated of these.

The *FMR1* gene product (*FMR1* protein or FMRP) exerts central and vital functions at the dendritic synaptic level, acting as both an organizer of messenger RNA (mRNA) transport (shuttle protein) and of translation of target mRNAs (RNA-binding protein), but also acting to downregulate its own local protein production. It is widely acknowledged that the X chromosome unsteadiness at Xq27.3 is caused by a dynamic mutation with a variable expansion of a trinucleotide (CGG) repeat [15]. The protein, like many found in human biology, also exerts an influence on reproduction and this is where our chief interests lie in this review.

The observed genetic instability in Xq27.3 seems to rely in the loss of AGG triplet inserts commonly identified every 9–

10 CGG repeats [23, 109–111]. These AGG interspersions (1 to 3 in normal and intermediate alleles, 0 to 1 in the premutated condition), accountable for allelic stability of the CGG expansion, may deeply influence the risk of full gene mutation and are also inherited (Fig. 1). In individuals carrying the same number of CGG repeats, such risk shows a 10-fold increase in those with loss of the AGG inserts. It is hypothesized that the AGG inserts perhaps act on the remodelling phenomena crucial for the modulation of neurosignals, as they limit the further expansion of the CGG repeats in subsequent generations [24]. In addition, AGG sequences share with the CGG repeats a level of instability, commonly the loss of an interruption during transmission to progeny [23]. Microsatellites and other types of triplet repeats probably contribute, during adaptive evolution, to species diversity and thus represent a central mutational event of great importance [54].

The CGG mutations

The extent and stability of the CGG repeat copy number affect the expression of diverse phenotypes through the classic four allelic forms of the *FMR1* gene [1]: (i) common (<45 CGG repeats), (ii) intermediate (45–54 repeats), (iii) premutated (55–200 repeats) and (iv) full mutated (>200 repeats) [93] (Table 1). No general agreement exists on the real amplitude

Fig. 1 Predicted risk of expansion during transmission by maternal total CGG repeat and AGG interruptions. Risk of expansion decreases when the number of AGG interruptions increases, for the same total CGG repeat length. The differential risk between one and two AGG interruptions is highest between 75 and 80 total CGG repeats. Taken and modified with permission from Yrigollen [109]

of each allelic form; therefore, the appraisal of data in the literature is sometimes cumbersome and facilitates interpretation errors. The vast majority of the Caucasian population exhibits a narrow CGG repeat range, peaking at 30. This reflects the work by Fu et al. [31] which demonstrated that unaffected individuals largely display 29–30 CGG repeats (Fig. 2). Although this finding may appear coincidental, 30 precisely matches to the quantity of repeats essential to generate a gene translation switching effect [19]. Nevertheless, recent investigations tend to set this cut-off point at a lower level. Allelic frequencies in the general population are quoted as 1:57 for the intermediate or ‘grey zone’ range (45–55 repeats) and 1:71 in the pre- and full-mutation range combined (>55 repeats). Interestingly, premature ovarian failure individuals have an *FMR1* mutation prevalence of 1:10 [20].

Additional extensive data from a newborn population screening study undertaken by Tassone et al. [93] elegantly shows the triplet repeat distribution across the full range of the potential expansion in an unselected group (Fig. 3). This data concurs with the earlier work by Fu et al. showing a large clustering between 18 and 42 repeats, with the majority between 27 and 32. A benefit of the Tassone data is a further analysis of the frequency of CGG repeats in the intermediate or grey area, and premutation range (Fig. 3b, d). Typically, <45 CGG repeats is considered normal [71]. Indeed, workers in the neurological field could not find any atypical phenotype association below 45 CGG repeats [87]. Some studies have shown, however, that reproductive function may be significantly impacted in this range [36]; however, other studies have shown no obvious impact [22, 58]. The American College of Obstetrics and Gynecology Genetics Committee, and the American College of Medical Genetics (ACMG), explicitly state that an *FMR1* CGG repeat length of <45 ‘is not associated with an abnormal phenotype’ [66]. This ‘normal’ range, however, may not in fact be a homogenous population. Gleicher et al. reported the intermediate genotype (45–54 repeats) expresses a minor instability unrelated to a definite phenotype or to the fragile X syndrome (FXS) within one generation, even though the risk is consistent in two generations [113]. These intermediate carriers, compared to ‘normal’, show a greater prevalence of subfertility, menstrual cycle anomalies, overall earlier than anticipated menopause (by ~7 years), premature ovarian failure (32 vs 1 %), increased prevalence of osteoporosis, higher rate of aneuploidy, miscarriage and even perhaps non-identical twinning rates [85, 94].

Repeats between 55 and 200 CGG characterize the premutation genotype. Even though carriers of premutation do not suffer from distinctive FXS symptoms, they may experience a rise in CGG repeat magnitude in ensuing generations that leads to the full mutation [107]. The premutation genotype has an incidence of 1 in 259 females, and of 1 in 810 in male individuals [23]. It

Table 1 The classic four allelic forms of the *FMR1* gene

Category	No. of repeats	Clinical relevance
Full mutation	>200	Fragile X mental retardation
Premutation	55–200	Risk of premature ovarian insufficiency
Intermediate	45–54	‘Grey Zone’ insufficient evidence
Normal phenotype	0–44	
‘High’ range	35–44	Potential risk of moderate ovarian dysfunction
‘Normal’ range	26–34	Normal ovarian reserve and function
‘Low’ range	19–25	Potential risk of significant ovarian dysfunction

is possible that prevalence may be influenced by ethnic or geographical factors. For example, an Israeli study identified a more frequent female premutation prevalence of 1 in 113 [95], while in Taiwan, a lower male incidence was observed at 1 in 1674 [97]. Ovarian dysfunction as may be found in the premutation group can manifest as premature ovarian insufficiency. While in full mutation carriers the risk for ovarian dysfunction (premature ovarian failure or amenorrhea before the age 40) is unchanged, in the premutation population, this risk reaches 16 to 20 % [91]. In the premutation population, data has shown that the relationship between CGG repeat numbers and ovarian reserve is non-linear ([26]). A peak in ovarian dysfunction is observed at 80–120 repeats, with intriguingly a lesser incidence of POI at higher repeat numbers [91].

Further evaluation of the premutation group may be useful as at least 80 % of these patients have unaffected ovarian reserve.

CGG repeats exceeding 200 copies define the full mutation and results in FXS in the majority of boys and in some girls, and the expansion observed in fact encompasses the gene’s promoter region. With this CGG repeat level, the *FMR1* gene is typically hypermethylated leading to transcriptional silencing and low or absent FMR protein levels [70]. At the embryonic stem cell level, the hypermethylation-coupled epigenetic silencing and subsequent lack of the *FMR1* protein does affect

the development and function of synapses [3] This sequence of events leads to the fragile X syndrome (FXS), where intellectual disabilities and an autism spectrum disorder manifests predominantly in males. In FXS, the alleles are highly unstable, through maternal transmission, and CGG repeat expansion often rises in the subsequent generations [98] (Fig. 1). The complete mutation with intellectual disability has a prevalence of 1 in 4000 and 1 in 6000, respectively, on males and females [96]. Remarkably, the number of CGG repeats in the *FMR1* gene tends to augment as the mutated gene passes from the mother to progeny.

How *FMR1* mutations influence different phenotypes

How the CGG repeat expansion regulates the *FMR1* gene expression and, therefore, the phenotype is still poorly understood. Once the number of CGG repeats is below 45, the production of mRNA and FMRP remains typically well-maintained, hence gross phenotype aberrations are not detected. Subtle changes in ovarian reserve and follicular activity, however characterize unbalanced repeats below 26 CGG’s and are thought to be due to decreased translation of FMRP (Fig. 4).

With intermediate (45–54 CGG repeats) and premutation carriers (55–200 CGG repeats), we notice a translation fault which can be held accountable for mRNA production levels

Fig. 2 Distribution of CGG repeats as opposed to the observed alleles in the general population. Taken and modified with permission from Fu [31] and Tassone et al. [93]

Fig. 3 CGG repeat allele size distribution. Histograms display the CGG repeat length observed in the newborn screening by allele category. **a** *FMR1* alleles in the normal range (<45 CGG repeats, $n = 20,710$ alleles). **b** *FMR1* alleles in the grey zone range (45–54 CGG repeats,

$n = 170$ alleles). **c** *FMR1* alleles in the expanded grey zone range (40–54 CGG repeats, $n = 614$ alleles). **d** *FMR1* alleles in the premutation range (55–200 CGG repeats, $n = 50$). Taken with permission from [93]

not balanced through an equivalent rise in FMRP that frequently is normal or somewhat reduced. By consequence, the mRNA levels build-up in the cells where it is hypothesized to exert cytotoxic effects (RNA/protein toxic gain-of-function disorder). Human *FMR1* mRNA in transgenic mice prompts premature ovarian insufficiency (POI) [60]. In this animal

model, the *FMR1* premutation does not play any role in early primordial follicles, but seems to act on subsequent steps of follicular maturation, as well as increasing the degree of follicular apoptosis. The mRNA toxicity hypothesis may be the underlying pathogenic explanation for the fragile X tremor/ataxia syndrome (FXTAS) (where neuronal and astrocyte

Fig. 4 Allelic distribution showing the relationship with *FMR1* mRNA and FMRP

intranuclear inclusions containing *FMRI* mRNA have been described), and possibly for fragile X premature ovarian insufficiency (FXPOI). Of great interest here also is the finding of ubiquitin-positive ovarian stromal cell intranuclear inclusions, detectable by immunohistochemistry [17] which resemble the neuronal inclusions seen in FXTAS individuals.

Lastly, reduced/absent mRNA and FMRP production account for the consequences of the complete mutation (>200 CGG repeats) where transcriptional methylation events and histone modification changes provoke complete gene silencing in FXS (loss-of-function disorder). Additionally, through the influence of AMPA receptor trafficking anomalies, the synapses weaken [2, 67]. Avitzour hypothesized that this gene silencing most probably occurs at the embryonic stem cell level [3]. (see Table 1). The *FMRI* gene polymorphisms may, in fact, cause a broad variety of medical disorders, including inherited intellectual disability, mood instability, behavioural quirks, learning difficulties, epilepsy, repetitive rituals and autism [47]. Classically, three distinct syndromes share the mutations in *FMRI* gene:

- (i) The fragile X syndrome (FXS). It is an X-linked dominant disorder with early onset, diminished penetrance and is considered as the prevailing cause of inherited intellectual disability [32]. Other disabilities appear sometimes associated, like intellectual and emotional issues, mood instability, learning difficulties and autism. Furthermore, specific phenotypical features may coexist, like macroorchidism, elongated face and uncommon hyperextensibility of fingers.
- (ii) The fragile X-associated tremor/ataxia syndrome (FXTAS). FXTAS is a neurodegenerative disorder (progressive cerebellar ataxia) clinically manifest with late onset in about 38 % of the male carriers of the premutation (55–200 repeats) (X-linked recessive disease), where the *FMRI* gene is entirely active [48]. The FXTAS occasionally presents as childhood seizures or early menopause. Thus, even though both FXS and FXTAS originate from a CGG repeat expansion of the *FMRI* gene, they express their clinical phenotypes at the two extremes of the age array.
- (iii) Premature ovarian insufficiency (POI). The menopause occurs on average at age 51, though normal inheritance, as investigated in twin and mother-daughter studies [63, 99]. POI, however, is a *continuum* disorder characterized by follicular dysfunction and/or depletion of primordial follicles before age 40, leading to infertility (~1 % of the population). This syndrome may contribute to the observed variances in menopause mean [91]. Once diagnosed, POI patients can have less than a 5 % chance for spontaneous pregnancy [7]. Individuals with POI typically exhibit a multiplicity of menstrual cycle conditions (from oligomenorrhea to amenorrhea),

atypically high levels of gonadotropins (>25 IU/L measured twice more than 4 weeks apart), low anti-Müllerian hormone (AMH) and oestrogen levels (and associated osteoporosis) [44]. Clinically, POI is found in ~15 to 21 % of female carriers of the premutation [57, 107].

The *FMRI* sub-genotypes within the normal range

There is some difference in opinion with regard to the interpretation of the normal range of *FMRI* gene due to fluctuations in the number of repeats. Out of the need for consistency when comparing *FMRI* gene mutation expansions in the population, grew the need for a re-interpretation of *FMRI* genetics, culminating in the reconsideration of the typical ranges of CGG repeats. The expected normal range has been suggested as between 26 and 34 repeats, rather than <44 [40]. It is possible that numbers outside of this could represent different sub-groups of the ‘normal’ population.

A proposed revised classification describes three separate genotypes:

- (i) Normal (*norm*, or *norm-norm*), when both alleles, or haplotypes, are in the 26–34 repeat range.
- (ii) Heterozygous (*het*), when only one allele is in the 26–34 repeat range. This comprises two sub-genotypes, the *het-norm/high* (second allele has >34 repeats) and *het-norm/low* (second allele has <26 repeats).
- (iii) Homozygous (*hom*), where both alleles are outside the proposed 26–34 range. This genotype consists of three further sub-groups: *hom-high/high* (both >34), *hom-high/low* (One allele >34 and the other <26) and *hom-low/low* (both <26).

Just as there is ethnic variation in the prevalence of the premutation genotype [95, 97], sub-genotypes may also differ. A study on IVF patients, with an analysis of patient ethnicity, noted that the frequency of normal range *FMRI* sub-genotypes varied between different racial groups [37]. African-American individuals expressed the *het-low/norm* allele more frequently than Asian patients, who, in contrast, showed a higher prevalence of *het-norm/high* alleles.

The *FMRI* gene impact on ovarian physiology

Our understanding of normal and pathological ovarian function has improved in recent years. There is evidence that the ovary ages following highly precise patterns (when compared to aging of other body organs and tissues), and that individual

variability profoundly impacts folliculogenesis and onset of menopause [94]. Normal ovarian function is, in fact, a *continuum* process that initiates early during intrauterine life and persists throughout reproductive life, up to menopause. Endocrinological [90], autoimmune [41] and iatrogenic factors [13], as well as genetic causes [72] and micro-environmental dynamics (i.e., endocrine disrupting chemicals) [45], are frequently described for their possible impact on the size and quality of the follicle and oocyte pool, and as potential causes of premature ovarian senescence. *FMR1* is one of the most commonly described genetic factors.

The number of human genes associated with POI/PO continuously grows [74]. Nevertheless, exactly how the *FMR1* gene influences ovarian physiology still remains a matter of investigation [85]. Substantial levels of *FMR1* protein have been found in the granulosa cells of growing follicles in normal women [81]. Interestingly, this study reported that the number of CGG tandem repeats did not influence the *FMR1* gene expression in individuals with premature ovarian failure [81]. Of interest also, is the finding that the CGG tandem repeats escape X chromosome inactivation in granulosa cells; therefore, both alleles are functional throughout folliculogenesis [73].

A recent suggestion that the accumulation of *FMR1* mRNA in granulosa cells is cytotoxic (toxic RNA gain-of-function model) may perhaps enlighten us as to how the low ovarian reserve observed in premutated carriers could be the pathogenetic mechanism for ovarian insufficiency [25]. Interestingly, the authors found that the number of retrieved eggs after IVF was non-linearly associated to the number of CGG repeats in the range of premutation, having an unfavourable prognosis in the range 80–120 that in turn was non-linearly associated with the highest concentration of mRNA in granulosa cells. A previous study has shown that the age of menopause did not appear to be a function of the size of the CGG repeats for the same 80–120 range [26].

An additional suggested pathogenetic explanation of the effect of this CGG repeat expansion mutation relies on the observed low rate of dehydroepiandrosterone (DHEA) conversion into testosterone (T) in women with low allele repeat numbers, as compared to others in the ‘normal’ range [104, 106]. A deficit of DHEA/T conversion has been identified in a subset of impaired ovarian reserve patients [34, 35].

DHEA supplementation prior to the stimulation phase of IVF in these patients with reduced ovarian reserve may induce a rise in serum testosterone, which some research has associated with an enhanced pregnancy rate [5, 59, 62, 106] while other work has shown no difference [53]. It is possible that one contributing factor to the lower pregnancy rates in this group of IVF patients may be an increased prevalence of the *heterozygous norm/low* allele genotype compared to the general population.

CGG mutations and biomarkers of ovarian reserve

The American Society for Reproductive Medicine in 2006 defined ‘ovarian reserve’ as the potential to achieve a healthy and successful pregnancy with respect to residual oocyte yield and quality evaluated at the moment of the investigation. Notwithstanding the extensive number of scientific papers in the last few years, no definitive consensus exists on the characterization of altered ovarian function [29]. A combination of hormonal markers like serum follicle-stimulating hormone (FSH) [52], serum AMH [79] and the antral follicle count [77] may help in diagnosing early decline in ovarian follicular activity. The antral follicle count remains perhaps the best predictor of the ovarian response to the controlled ovarian stimulation after the AMH assay itself [80, 88]. Although early follicular phase serum FSH levels are a well established marker of ovarian reserve [18], the correlation may be poor compared to other markers. Moreover, its relationship to the decline of the ovarian activity often appears later in life. AMH, on the contrary, represents the gold standard in the appraisal of the residual follicular pool [88], probably because of its role in governing small growing follicle development and recruitment [101]. In contrast with assessing FSH as a marker of ovarian reserve, AMH has certain advantages: (a) production occurs in the granulosa cells of small primordial follicles, (b) it is not cycle-day specific and (c) it appears raised in individuals with PCOS. Prior to the advent of automated platform testing, AMH testing suffered from variability of results due to assay and storage variability [64].

The relationship between ovarian aging and the X chromosome share a long history [9, 76, 92]. Scientists have discovered a considerable number of genes and candidate genes on both X chromosome arms, that in many ways link strongly the X chromosome to human reproductive capability [49, 50]. The existing association between the *FMR1* gene, in the premutation range (previously not considered a risk for any medical condition) and primary ovarian insufficiency (POI), is acknowledged by many authors [46, 107]. However, in 2009, the work of Gleicher and colleagues confirmed that the length of the CGG repeats, primarily in the ‘new’ normal range, paralleled the FSH and AMH values, and similarly the clinical picture [39]. Gleicher studied how the number of CGG repeats compares with the relative risk of having an AMH value below 0.8 ng/ml [38]. Remarkably, below and above 30 CGG repeats, this risk seemed significantly amplified either in young or older women. CGG repeat number is also important as evidenced by the observation that for every five CGG repeats above or below 30 we observe a rise in relative risk of having a pathologic AMH value by, respectively, 60 and 40 % [38].

For decades, the *FMR1* repeat expansion, within the premutation range (55 to 200 repeats), was the cause of

premature ovarian failure. As previously expressed in this paper, full mutated individuals do not exhibit a substantial increase in premature ovarian failure (POF) a prevalence that, in contrast, characterizes the premutated women. The *FMRI* gene premutation has a prevalence of 11.5 % in the presence of familial premature ovarian failure (POF), as compared to 3.2 % found in families with sporadic POF [16].

In 2014, De Geyter published some discordant results [22]. The author, in fact, presented in his paper the first prospective study done on infertile individuals and on women with proven fertility. The author studied 199 patients who delivered within 3 months and compared them with 372 normally menstruating infertile women and 48 individuals with POI. Among the three mutation ranges investigated (premutation, intermediate and high normal) none showed a statistically significant association with infertility nor with ovarian reserve indicators. Slightly earlier, Lledo et al. also published results on the use of *FMRI* screening in young oocyte donors in predicting the ovarian follicular development [58]; numerous papers on the other hand report a substantial link between *FMRI* mutations and ovarian reserve [11, 33, 86].

***FMRI* gene mutations and reproductive aging**

The aetiology of premature ovarian failure remains unknown in many cases, with some exceptions, such as Turner's syndrome, or as a result of medical interventions in oncology (chemotherapy, radiotherapy, etc.). Suggested mechanisms of ovarian insufficiency rely on either a decreased oocyte pool [12], augmented follicular atresia [51, 112] and/or altered folliculogenesis [78]. The clinical presentation of ovarian insufficiency varies significantly. Serum FSH level and the menstrual cycle may appear unmodified in occult ovarian insufficiency, in which case only a reduction in fertility may coexist. A rise in serum FSH levels with still regular menstrual cycles embodies diminished ovarian reserve (DOR), and fertility is impaired. Menstrual cycles become irregular or absent and serum FSH levels rise in response to increased pituitary GnRH release characterized by compromised fertility [68].

In recent years, the list of potential gene candidates for ovarian aging on the autosomes and on the X chromosome itself has continuously grown. The study of the real impact of each gene and mutations on ovarian physiology requires great effort [102, 103]. Nevertheless, the results of these genetic studies have been rather cumbersome, requiring new investigations in order to significantly increase the chances of finding strong gene/phenotype associations.

It has been suggested that the death of granulosa cells and perhaps oocytes themselves in POI and POF can recognize, as a pathogenic mechanism, a dynamic intracellular accumulation of cytotoxic amounts of aggregated CGG repeats that in

turn sequester proteins like DROSHA and Sam68 [83]. The final result will be the interference with some important cellular functions.

A cross-sectional study evaluated the infertility traits and pregnancy rates in normal women and in individuals carrying the premutation and found that the premutations are in a non-linear relationship with POI [1]. In this paper, based on questionnaire data from 948 patients, the author categorized the individuals depending on the CGG repeat size: below 59 CGG repeats patients were non-carriers; low premutation occurred from 59 to 79 repeats; mid premutation extended from 80 to 100 repeats and finally high >100 repeats. The mid-size CGG repeats group had an earlier onset and a higher prevalence of POF as compared to the other groups, while the miscarriage rate was unmodified.

Bodega studied a large cohort of patients ($n = 190$) showing premature ovarian failure in association with size, sequence, and X-inactivation [8]. This study strengthened the bond between CGG size and ovarian dysfunction, but also stated that the pattern of interruption of the repeats and X-inactivation may negatively affect the ovarian physiology.

In 2009, Streuli et al. published a paper on a study conducted on 27 patients with low ovarian reserve and 32 controls, setting the intermediate mutation range at 41–60 CGG repeats [89]. In the group of patients with low ovarian reserve, the mean size of CGG repeats was 33, while in controls it was 28. *FMRI* alleles with more than 40 CGG repeats (intermediate and premutated) had occult POI (22 % of the patients), while in controls this value reached the 3 %. The authors concluded that women with signs of occult POI demand careful investigations as they may hide CGG repeat expansions in the intermediate and premutation range.

Gleicher reported at least two specific geno-/phenotypes in premature ovarian aging:

(i) The 'genetic' type

- Not associated with autoimmunity
- Obesity related
- Hyperandrogenic
- Characterized by severe ovarian dysfunction (high FSH and low AMH)
- Commonly associated with the conventional POI

(ii) The 'autoimmune' type

- Associated with autoimmunity
- Non obesity related (typically observed in lean PCO-like individuals)
- Non hyperandrogenic
- Characterized by a milder ovarian dysfunction (lower FSH and higher AMH)
- Strongly associated with *het-norm/low*

- Rapidly depleting ovarian reserve
- Decreased pregnancy rate (PR) after IVF

The *FMR1* gene and the proximity to immune-related genes

Several investigations have highlighted the prominence of the long arm of the X chromosome [14], since it maps, alongside the *FMR1* and POI genes, for the highest number of mutated or polymorphic genes in the genome. In fact, these ‘modified’ genes seem to play crucial roles in selected immune-related medical illnesses [6, 72].

Autoimmune diseases are more prevalent in the female and this has prompted for a possible role for the X chromosome in autoimmune pathogenesis [27, 28, 69]. Some autoimmunity-related genes on the X chromosome are indeed well characterized, for instance, a chain reaction triggered by a product of the *IRAK1* gene mapped at Xq28 activates the innate immune response. *CD40LG* gene in Xq24, which codes for a membrane protein expressed by activated CD4+ T cells (adaptive immune response), is located on the X chromosome and associated with autoimmunity [4]. *FOXP3* gene at Xp11.23 is a transcription factor modulating the activity of Treg cells [55] which are key modulators of the activity of other effector T and NK cells. Furthermore, Shamilova found that levels of anti-ovarian antibodies >10 IU/ml in women affected by POF positively associate with the number of CGG repeats [84].

Recently, multiple regression analysis has reinforced the hypothetical link existing between the *FMR1* gene and autoimmunity. Indeed, a statistically significant association seems to join the *FMR1* genotypes/sub-genotypes, autoimmunity and the likelihood of IVF pregnancy [36] (Fig. 6).

Controversial ‘protective’ role of *het-norm/high* requires further investigations, since the observed differences did not reach statistical significance. Schufreider conducted a retrospective data analysis on 1287 patients who underwent testing for CGG repeats and ovarian reserve (day 2 or 3 FSH, AMH and antral follicle count) [82]. In this study, no distinction was made between women with one or two high normal alleles. Another potential study limitation was that patients were not stratified according to their infertility diagnosis. The author concluded that patients can be reassured that a *het-norm/high* genotype is not responsible for diminished ovarian reserve. Unfortunately, in this paper, the author used, as his reference range, *het-norm/high* repeats of 35–54 [82]. Autoimmunity and CGG expansion size were again related, reinforcing the importance given to the non accidental topographical relationship among CGG triplet repeats and autoimmunity loci on the X chromosome. Indeed, the prevalence of autoimmunity was

higher (51 %) in PCO-like phenotype with *het-norm/low* ($p = 0.0001$). Autoimmunity in *het-norm/high* was lower (10 %) than in *norm* (24 %) indicating a possible protective effect of this sub-genotype. The pregnancy rate in this study in Caucasians was higher than in Africans. It remains unknown as to why and how specific sub-genotypes apparently influence IVF pregnancy rates in different races.

The prominence of the *het-norm/low* allele

Since the *FMR1* gene was recognized as accountable for the fragile X mental retardation syndrome, the research has been centred primarily on full mutation carriers. Attention soon turned to expanded repeat sequences in the premutation range (55–200 repeats) and below. Rapidly growing evidence of the literature is accumulating, and the short CGG sequences comprised in the normal distribution curve are increasingly dominating the stage.

The work of Gleicher, with the advocated revision of the CGG repeat expansion classification, has pointed to the effect of the short expansions, at the other end of the spectrum below 26 CGG repeats. Mailick in 2014 also added new proof of the importance of short CGG sequences in individuals with 23 or fewer repeats. The author compared these patients (older adults) to a group of age-matched individuals with a number of repeats within the normal range (24–40 repeats) [61]. The main measures of this retrospective study were cognition, behavioural functioning, neoplasia and presence of children with mental health issues. This study analyzed a sample of individuals extrapolated from the Wisconsin Longitudinal Study, comprising men ($n = 341$), with the *het-norm/low* mutation, and women ($n = 46$) with two *norm/low* alleles. Individuals with sub-genotype *het-norm/low* exhibited some strain in memorizing or solving daily issues compared to *norm* individuals ($p < 0.05$). Anxiety and depression, however, were not linked to CGG size. Curiously, women with *het-norm/low* revealed that they needed to drink more than in the past to calm their thirst and perceive the equivalent effect ($p < 0.001$). A statistically significant association with breast cancer arose in women *het-norm/low*, presenting also with increased odds of having had uterine cancer in the past ($p = 0.074$). Carriers of *het-norm/low* CGG mutations have had in their life a child with some level of disability as compared to *norm* individuals ($p < 0.05$). Certainly, these data warrant additional investigations in more significant population sample sizes, since some of the detected outcomes (disability in children, cognition problems, depression, bipolar disorders, etc.) may be interrelated and due to the occurrence of other concomitant variables. Instead, a direct impact of low CGG repeats on these outcomes cannot be ruled out. On the other hand, as proposed by some authors, a low number of CGG repeats supposedly

rescued embryos with *BRCA1/2* mutation [42, 105], even though this association has been refuted by others [10, 21].

The importance of the *het-norm/low* CGG polymorphism as a screening tool with the ability to recognize individuals at risk of premature ovarian aging is becoming more widely accepted. Recently, Gleicher published the results of the first longitudinal cohort study conducted on 233 highly selected, healthy oocyte donors [43]. In addition, the authors investigated a subset of this cohort ($n = 66$), looking at those donors with more than one AMH determination. The AMH value correlated with the presence or absence of specific *FMR1* sub-genotypes. In fact, *hom-low/low* donors revealed, since their preliminary investigations, a noticeable decrease in ovarian reserve ($p = 0.001$). In contrast, *het-norm/low* women displayed a normal ovarian reserve at their early investigations, nonetheless 4 years later the ovarian reserve significantly declined ($p = 0.046$). Both *hom-low/low* and *hom-high/high* associated with a substantial drop in ovarian reserve (revealed by reiterated AMH determinations), as compared to donors lacking *FMR1* gene mutations. This study proposes that women showing *low* alleles experience a loss of their reproductive potential (oocytes) at a faster rate as compared to individuals not showing such a *FMR1* sub-genotype.

The *FMR1* gene and IVF outcome

Extensive research has been performed on the link between *FMR1* gene mutations and ovarian physiology. Premature ovarian insufficiency has been shown to present in a significant proportion of patients with the premutation; however, additional research has suggested that premature ovarian dysfunction may be linked to specific *FMR1* sub-genotypes in the ‘normal’ range. With increasing use of assisted reproductive technologies, further research was required to assess the potential impact of *FMR1* gene expansion mutations on outcomes of in vitro fertilization (IVF) programs. In particular, one area of study has been to assess the possible role of *FMR1* genotypes as a prognostic marker of outcome in reproductive medicine. In 2014, Kushnir conducted a large investigation on the possible impact of various *FMR1* genotypes and sub-genotypes on morphological embryo quality in 125 infertile patients (168 IVF cycles and 777 embryos); on 149 IVF cycles (1041 embryos) in potentially fertile women to evaluate ploidy, and on 352 young and infertile women and 179 oocyte donor/recipient cycles to assess the IVF outcome [56]. A low *FMR1* sub-genotype (29.6 % of the patients) impacted negatively the embryo morphology and the pregnancy rate despite of the age of the mother. Embryo morphology was impaired in a statistically significant manner also in sub-genotypes *hom-low/low* and *hom-low/high*. Ploidy, as expected, increases with patient age but showed no

association with a specific *FMR1* gene sub-genotype, although the high prevalence of aneuploidies in *hom-low/low* requires further investigations. Oocyte donor age or donor *FMR1* status did not influence recipient pregnancy rate. Although it appears to have an influence on serum AMH levels (Fig. 5). Also, difficult to explain is the finding that in *hom-high/low* the percentage of embryo aneuploidies is approximately 50 % of that found in *norm*. This finding reinforces the idea that embryo morphology is often an obsolete way to predict ploidy. In donated oocyte recipients, no significant association was found between donor genotypes and clinical pregnancy rates, while in middle-aged patients statistical significance was again found in sub-genotypes showing at least one allele *low* [56]. An earlier study has similarly demonstrated diminished IVF outcomes linked to specific normal range sub-genotypes, with *het-norm/low* patients displaying poorer PR as compared to *norm* ($p = 0.001$) (Fig. 6). Interestingly, after adjustments were made for age, disadvantages in pregnancy rates for *het-norm/low* patients were maintained (OR 0.43, $p = 0.017$) [36]. The potential repercussions of this finding could be that *low-norm* genotype women may require more treatment cycles to achieve success, with additional financial and time implications, and this may need to be considered during patient counselling.

Preimplantation genetic diagnosis

Preimplantation genetic diagnosis (PGD) is an increasingly important tool in the hands of the IVF physician, particularly given the unusually high aneuploidy rate seen in females above the age of approximately 37 years [30]. Given that the majority of *FMR1* patients in the grey zone or premutation range are not necessarily aware of their condition and will not present to the IVF clinic with primary ovarian dysfunction, it is hard to consider the benefit of PGD screening in these cases. The potential to inhibit the risk of full allelic expansion to the

Fig. 5 Graphical representation of the relationship between declining AMH, *FMR1* allele status and age

Fig. 6 Pregnancy rates with IVF across the normal range sub-genotypes. (direct from Gleicher et al. [36], open access permission)

offspring, however, is a desirable outcome [108]. On a practical level, recent studies have indeed demonstrated the feasibility of performing PGD on embryos from FMR-1 affected females with alleles in the premutation range. The use of PGD has shown that fragile X carriers transmit the abnormal allele to embryos at the same frequency as the normal allele [75].

Final considerations

We know that the *FMR1* gene CGG expansion mutations seem to control the ovarian physiology and, by consequence, dictate the age of the menopause onset, but still how these mutations act remains enigmatic. Among the revised *FMR1* gene sub-genotypes, the *het-norm/low* CGG expansion unquestionably negatively influences the success of both in vivo and in vitro human reproduction, acting in a still poorly understood mode on ovarian physiological functions. These subtle genetic abnormalities induce the appearance of progressively severe conditions that range from occult ovarian insufficiency in young women to premature ovarian failure, through a complete spectrum of diverse hormonal and phenotypical changes. In addition, the *het-norm/low* sub-genotype may delineate an autoimmune-related, rapidly ovarian-reserve depleting polycystic ovary phenotype.

Some social and behavioural shifts (including the so-called reproductive delay) and the availability of enhanced techniques applied to human reproduction have drastically increased the demand for female fertility testing. The *FMR1* gene, assessed by a relatively simple blood test, may indicate to scientists valuable information about a woman's fertility and may help young women in discovering, in the early phase of their reproductive life, potential ovarian reserve problems later in life. All this should let us properly counsel women and tailor infertility treatments with a little more care, using a

parameter that most people usually ignore. This said, the intriguing role of the *FMR1* gene in neurophysiology and reproduction requires larger epidemiological studies and experimental research before it may be admitted to mainstream diagnosis.

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