

·Original Article·

## Erectile dysfunction in Fragile X men

Feng Gu<sup>1,2</sup>, Hai Y. Zhang<sup>3,4</sup>, Shao Y. Hu<sup>2,5</sup>, Shang Z. Huang<sup>2,5</sup>, Xu Ma<sup>1,2,6</sup>, Yong Q. Zhang<sup>3</sup>

<sup>1</sup>Department of Genetics, National Research Institute for Family Planning, Beijing 100081, China

<sup>2</sup>Peking Union Medical College, Beijing 100081, China

<sup>3</sup>Key Laboratory of Molecular and Developmental Biology, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100080, China.

<sup>4</sup>Graduate School, Chinese Academy of Sciences, Beijing 100080, China.

<sup>5</sup>Department of Medical Genetics, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, Beijing 100005, China

<sup>6</sup>Department of Reproductive Genetics, WHO Collaborative Center for Research in Human Reproduction, Beijing 100081, China.

### Abstract

**Aim:** To further characterize the sperm defects in Fragile X men. **Methods:** Two different polymerase chain reaction (PCR) based methods were used for the molecular diagnosis of FXS. Sperm collection was done mostly according to the laboratory manual of the World Health Organization. **Results:** We failed to collect sperm samples from five Fragile X subjects aged 18–60 years as a result of an unexpected erectile dysfunction. **Conclusion:** Erectile reflex is an instinctive response in all healthy males. The absence of erection can be caused by hormonal, physical or neuronal malfunction. As hormonal profiles are reported to be generally normal in Fragile X men, we propose that an unknown physical factor or the neuronal circuit, or both, underlying the erection is compromised. (*Asian J Androl* 2006; :)

**Keywords:** Fragile X syndrome; *FMR1* gene; macroorchidism; erection; fertility; erectile dysfunction

### 1 Introduction

Fragile X syndrome (FXS) is the most common form of inherited mental retardation worldwide [1]. It is caused

Correspondence to: Dr Yong-Qing Zhang, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, 3 Nan-Yi-Tiao, Zhong Guan Cun, Beijing 100080, China.

Tel: +86-10-6255-5190, Fax: +86-10-6255-1951

E-mail: yqzhang@genetics.ac.cn

Or Dr Xu Ma, Department of Genetics, National Research Institute for RFamily Planning, Beijing 100081, China.

Tel: +86-10-6217-6870, Fax: +86-10-6217-9059

E-mail: genetic@263.net.cn

Received 2005-12-07 Accepted 2006-02-28

by an expansion of CGG repeats in the 5' regulatory region of the Fragile X Mental Retardation 1 (*FMR1*) gene, leading to hypermethylation and subsequent transcriptional silencing of *FMR1* and the absence of its coding protein FMRP. There are two hallmarks of FXS: mental retardation and macroorchidism; that is, enlarged testicles after puberty, consistent with the finding that FMRP are highly enriched in brain and testes. At least 83% of postpubertal FXS men show macroorchidism [1]. Extensive research has focused on the neurological aspects of FMRP, but its role in testes development and/or spermatogenesis remains largely unknown.

Although FXS men have been reported to be fertile,

their offspring have rarely been documented [2–6]. A putative spermatogenesis defect was first reported in men with macroorchidism nearly 3 decades ago [7]. Later, Johannisson *et al* [8] reported that the early stages of spermatogenesis in FXS men were normal but the later stages were defective with significantly malformed spermatids and a reduction of normally differentiated spermatids, which might cause reduced fertility. However, the fertility of FXS men is still an open question.

The *FMRI* knockout mice established by an international consortium also displayed prominent macroorchidism [9]. However, the litter size of knockout mice was normal and initial light microscopic analyses suggested normal spermatogenesis [9]. Nevertheless, late-stage spermatogenesis defects can escape scrutiny at the light microscopic level using standard histological analyses of testicles, and sperm counts as low as 30% of normal are known to produce normal litter sizes [10]. Our recent examinations of both knockout mice and *Drosophila* mutants demonstrated that late stages of spermatogenesis were defective in the two animal models. In the case of knockout mice, obviously malformed spermatids were observed with sperm counts severely reduced; in the fly mutants, no motile sperm were found in the seminal vesicles [11, 12].

We wished to examine the sperm morphology and function of adult FXS men. To our surprise, we failed to obtain sperm samples from all five FXS subjects that we examined with standard sperm collecting procedures, as a result of unexpected erectile dysfunction. The finding of erectile dysfunction in FXS subjects will help us better define its clinical spectrum and pathogenesis of FXS.

## 2 Materials and methods

### 2.1 Subjects

Five mentally retarded subjects, A (18 years), B (20 years), C (19 years), D (18 years) and E (60 years), were studied in this study. The main physical features of the five subjects included prominent ears (subjects A, B, C and E), hyperextensible finger joints (all subjects), macroorchidism (all bilateral except C, who had unilateral macroorchidism), high-arched palate (subjects A and C), single palmar crease (subject B), flat feet (subject D) and strabismus (subject A).

All of them showed similar behavioral symptoms including hyperactivity, hand biting (subject B showed obvi-

ous hand and arm calluses), repetitive jocular speech, and autistic-like behavior, such as poor eye contact and attention deficit. Individually, subject B uttered incomprehensible, random words at infrequent intervals; subject C had difficulty in standing. Subjects A, B and C were from the same institution in Liaoning Province, China; subjects B and C were cousins (Figure 1, family I). Subjects D and E were from Shandong and Henan Province, respectively. Subject E had a deceased uncle who showed similar clinical signs (Figure 1, family II). No family history was available for subjects A and D.

Complete informed consent was obtained from their parents and/or legal guardians for all five subjects. An institutional Ethical Committee approval was granted to this study and all interventions performed were in accordance with the Helsinki Declaration.

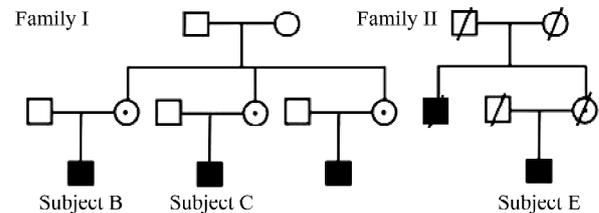


Figure 1. The pedigrees of two Fragile X syndrome (FXS) families. A square represents a man; a circle a woman; a circle with a center dot female carrier. A forward slash indicates deceased; a filled square an FXS man.

### 2.2 Polymerase chain reaction based methods

Two different polymerase chain reaction (PCR) based methods were used for the molecular diagnosis of FXS. One was a simple PCR method to measure the number of CGG repeats, based largely on the previously published protocols [13]; the other was designed to detect the methylation status of the 5' untranslated regulatory region (UTR) of *FMRI*. In general, methylation is associated with a full mutation, whereas premutation and the normal allele of *FMRI* show no methylation in the 5' UTR. The methylation sensitive PCR of the 5' UTR of *FMRI* was performed following a previous protocol with minor modifications [14]. Briefly, DNA samples from blood cells were first digested to completion with DNA

restriction enzyme **EagI** [Q1], and then used as templates for PCR with primers FRAX-g (5'-AGTGCACCTGT-CACCGCCCTTCAGCCTTC-3') and FRAX-h (5'-GAAACCACGTCACGTGATCAACGCTGTTCC-3') flanking the restriction site. Amplification products were subjected to electrophoresis on a 6% polyacrylamide gel followed by silver staining. To monitor the PCR reaction, a pair of primers (5'-CTGGCACCTATGGACACG-3' and 5'-GTCTTCTGGGTGGCAGTGAT-3') giving rise to a 367 bp band of the house-keeping **GAPDH** gene was included in the PCR system as the internal control.

### 2.3 Sperm collection

Sperm collection was done mostly according to the laboratory manual of the World Health Organization [15]. Briefly, **stimulation** was performed by self-**masturbation** and assisted-masturbation in a private environment.

### 3 Results

Based on physical and behavioral manifestations, including mental retardation, macroorchidism, and family history in the cases of subjects B, C and E, we suspected that these five subjects suffered from FXS. Two PCR based molecular analyses confirmed the diagnosis. The first test showed that the normal size of fewer than 50 CGG repeats was absent in all five subjects (data not shown). The second test demonstrated that the 5' UTR around the CGG repeat are hypermethylated, and therefore cannot be cut by a restriction enzyme **EagI**, which recognizes the unmethylated sequence, thus producing a PCR amplification band spanning the region (Figure 2, lanes 2, 4, 6, 8, 10 and 12). However, the normal allele of *FMRI* can be cut by **EagI**, therefore producing no positive PCR band spanning the region (Figure 2, lane 14). As an internal control, PCR reaction for house-keeping gene encoding **GAPDH** was **positive** (Figure 2, lanes 14, 15). Taking the clinical profiles and molecular analyses together, we concluded that the five subjects had FXS.

According to the published literature over the past 3 decades, including our own recent studies [8, 12], we reasoned that **FMRP** played a role in testes development and/or spermatogenesis. In an attempt to study spermatogenesis in FXS subjects, we tried to collect sperm samples from the five subjects by masturbation (self and

assisted), **private** setting, but failed in all of them, as a result of **unexpected** erectile dysfunction. Multiple examinations of the same subject at different times, and of different subjects from different provinces examined by different physicians, showed the same result consistently in all five subjects. As a control, subjects with **down** syndrome and other subjects with unknown conditions housed in the same institution showed robust erection in the same setting. In agreement with the above observation, long term caretakers in the institution observed in a time frame of several years that FXS subjects showed no erection whereas others did when taking a shower. Erectile dysfunction in FXS subjects was not a result of any noticeable physical deformity, as pubic hair and penis development of the five FXS subjects were normal.

### 4 Discussion

FXS men have been reported to be fertile [5, 6, 16]. However, our earlier work and studies by others on FXS men and animal models indicate that **FMRP** plays a role in spermatogenesis [8, 12]. Therefore, we sought to re-evaluate the morphology and function of sperm from FXS subjects. To our surprise, our work showed that all five FXS subjects examined had erectile dysfunction.

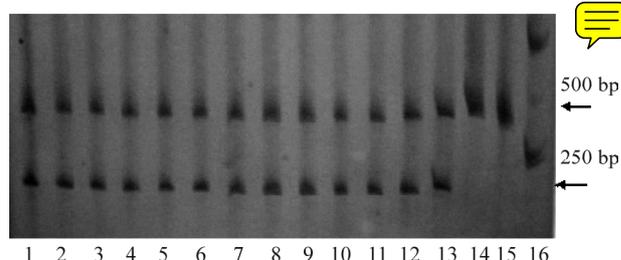


Figure 2. Polymerase chain reaction (PCR) analysis of the methylation status of the *FMRI* 5' UTR region. Lanes 1–10 were samples from subjects A–E (two adjacent lanes for each subject); lanes 11–12 from a subject previously confirmed as having Fragile X syndrome (FXS) by Southern blot; lanes 13–15 from a normal man; lane 16 molecular size marker. Odd numbers from 1 to 13 were genomic DNA without restriction cut; even number from 2 to 14 genomic DNA cut with **EagI** before the PCR reaction; lane 15 was an internal PCR control for glyceraldehyde-3-phosphate dehydrogenase (**GAPDH**) only. The upper band, shown by an arrow, was specific for a **GAPDH** gene fragment of 367 bp; the lower band of 215 bp was specific for the 5' UTR spanning the **EagI** site of *FMRI*. All samples showed the upper control band (lane 1-15). All **subjects** showed the *FMRI* specific band irrespective of whether the DNA was cut by **EagI** (lanes 1–12), whereas a normal male did not show the lower band when the DNA was cut by **EagI** (lane 14).

The differences of reproductive ability among FXS men might be caused by sampling difference. The five subjects we report here were probably very severe cases, as they showed a full pattern of FXS symptoms and stayed in a local institution supported by the government. The discrepancy is consistent with the fact that the clinical spectrum of FXS subjects is wide: as in other cases of mental retardation, some are mild, others severely retarded [1]. The wide spectrum of clinical profiles including reproductive ability of FXS subjects might result from: (i) unidentified genetic background and/or (ii) mosaicism in the methylation status of the CGG repeat region, or the size of the CGG repeat, or both which has been documented in many cases [17–19]. It is well established that one special form of “size mosaicism” of late stage sperm cells always contain a premutation, whereas peripheral lymphocytes contain a full mutation [3, 4, 16]. Indeed, the FXS men recently documented to be fertile were mosaic of *FMR1* mutations [5, 6, 16].

Although macroorchidism is a hallmark of FXS, there is no published report, as far as we know, of successfully collecting sperm through a non-invasive approach from FXS subjects, except for the few fertile mosaic cases [5, 16]. However, biopsies of testes have been performed to examine spermatogenesis and mutation status in the sperm cells of FXS subjects [4, 8]. Considering that sperm collection is a simple, routine technique for clinical evaluation of reproductive ability, the fact that they went so far as to use biopsies of testicles indicates that they might also have encountered the same problem of erectile dysfunction, as we present here. Many conditions can cause erection dysfunction [20]. The absence of erection suggests that hormonal, physical and/or neuronal factors involved in the erection are defective. Although we cannot formally rule out the possibility of a hormonal alteration causing the erectile dysfunction in the five FXS men examined, it has been reported that an array of sex hormones in 15 FXS males was normal [21], as well as in another four mental retarded subjects with macroorchidism [7]. The same was true for follicle-stimulating hormone in the FXS mouse model [22]. As neurobehavioral and connective tissue abnormalities are common in FXS men, we propose that the neuronal circuit and/or an uncharacterized physical defect underlying the erection are compromised. It is worth noting that FXS men are hyperarousal and hyperactive [1], but the erection function is lost in at least some FXS men, as presented here. The finding of this symptom in FXS

subjects might help us better understand the clinical spectrum and pathogenesis of the disease.

We do not know why the erectile dysfunction in FXS men has escaped the physician’s scrutiny in the past several decades since FXS was firmly diagnosed in the late 1970s [23], nor do we know if the dysfunction is specific to the Chinese population. It will be of great interest to determine the percentage of FXS men showing erectile dysfunction and to what extent erectile dysfunction is associated with macroorchidism and the absence of FMRP. Based on our experience that all five subjects examined had erectile dysfunction, we suspect that the symptom might be prevalent among FXS subjects. As macroorchidism is widespread in many other forms of mental retardation, it will be of interest to examine erection function in other mental diseases, particularly in those with macroorchidism.

#### Acknowledgement

We thank the subjects, families and local institutions for their cooperation in this project. Dr Lin Wang participated in the early stage of this project. We are grateful to physicians Shi G. Han, Xiao Zhang and Li M. Song for assistance in the clinical examinations. We are indebted to our colleagues, Drs Wei Li, Zhao-Hui Wang and Jian Zhang, for discussions and comments on the manuscript. This work is supported, in part, by the National “973” Basic Research Funding Scheme of China (grant number 2001CB5103) and the National Infrastructure Program of Chinese Genetic Resources (2004DKA30490) to Dr Xu Ma, and grants from the Chinese Academy of Sciences and the National Natural Science Foundation of China (30430250, 30525015) to Dr Yong-Qing Zhang.

#### References

- 1 Hagerman RJ. The physical and behavioral phenotype. In: Hagerman RJ and Hagerman PJ, editors. *Fragile X Syndrome, Diagnoses, Treatment and Research*. Baltimore: The Johns Hopkins University Press 2002; p3–109.
- 2 Jacobs PA, Glover TW, Mayer M, Fox P, Gerrard JW, Dunn HG, *et al*. X-linked mental retardation: A study of 7 families. *Am J Med Genet* 1980; 7: 471–89.
- 3 Malter HE, Iber JC, Willemsen R, de Graaff E, Tarleton JC, Leisti J, *et al*. Characterization of the full fragile X syndrome mutation in fetal gametes. *Nat Genet* 1997; 15: 165–9.
- 4 Reyniers E, Vits L, De Boulle K, Van Roy B, Van Velzen D, de Graaff E, *et al*. The full mutation in the FMR-1 gene of male fragile X patients is absent in their sperm. *Nat Genet* 1993; 4:

- 143–6.
- 5 Rousseau F, Robb LJ, Rouillard P, Der Kaloustian VM. No mental retardation in a man with 40% abnormal methylation at the FMR-1 locus and transmission of sperm cell mutations as premutations. *Hum Mol Genet* 1994; 3: 927–30.
  - 6 Willems PJ, Van Roy B, De Boule K, Vits L, Reyniers E, Beck O, *et al.* Segregation of the fragile X mutation from an affected male to his normal daughter. *Hum Mol Genet* 1992; 1: 511–5.
  - 7 Cantu JM, Scaglia HE, Medina M, Gonzalez-Diddi M, Morato T, Moreno ME, *et al.* Inherited congenital normofunctional testicular hyperplasia and mental deficiency. *Hum Genet* 1976; 33: 23–33.
  - 8 Johannisson R, Rehder H, Wendt V, Schwinger E. Spermatogenesis in two patients with the fragile X syndrome. I. Histology: Light and electron microscopy. *Hum Genet* 1987; 76: 141–7.
  - 9 Bakker CE, Verheij CE, Willemsen R, van der Helm R, Oerlemans F, Vermeij M, *et al.* Fmr1 knockout mice: A model to study fragile X mental retardation. *Cell* 1994; 78: 23–33.
  - 10 Schurmann A, Kolling S, Jacobs S, Saftig P, Krauss S, Wennemuth G, *et al.* Reduced sperm count and normal fertility in male mice with targeted disruption of the ADP-ribosylation factor-like 4 (Arl4) gene. *Mol Cell Biol* 2002; 22: 2761–8.
  - 11 Zhang YQ, Broadie K. Fathoming fragile X in fruit flies. *Trends Genet* 2005; 21: 37–45.
  - 12 Zhang YQ, Matthies HJ, Mancuso J, Andrews HK, Woodruff E 3rd, Friedman D, *et al.* The Drosophila fragile X-related gene regulates axoneme differentiation during spermatogenesis. *Dev Biol* 2004; 270: 290–307.
  - 13 Erster SH, Brown WT, Goonewardena P, Dobkin CS, Jenkins EC, Pergolizzi RG. Polymerase chain reaction analysis of fragile X mutations. *Hum Genet* 1992; 90: 55–61.
  - 14 Dobkin C, Ding X, Li S, Houck G Jr, Nolin SL, Glicksman A, *et al.* Accelerated prenatal diagnosis of fragile X syndrome by polymerase chain reaction restriction fragment detection. *Am J Med Genet* 1999; 83: 338–41.
  - 15 World Health Organization laboratory manual for the examination of human semen and sperm-cervical mucus interaction, 4th edn. Cambridge: Cambridge University Press; 1999.
  - 16 Zeesman S, Zwaigenbaum L, Whelan DT, Hagerman RJ, Tassone F, Taylor SA. Paternal transmission of fragile X syndrome. *Am J Med Genet A* 2004; 129: 184–9.
  - 17 Nolin SL, Glicksman A, Houck GE Jr, Brown WT, Dobkin CS. Mosaicism in fragile X affected males. *Am J Med Genet* 1994; 51: 509–12.
  - 18 Rousseau F, Heitz D, Tarleton J, MacPherson J, Malmgren H, Dahl N, *et al.* A multicenter study on genotype-phenotype correlations in the fragile X syndrome, using direct diagnosis with probe StB12.3: the first 2,253 cases. *Am J Hum Genet* 1994; 55: 225–37.
  - 19 Schmucker B, Seidel J. Mosaicism for a full mutation and a normal size allele in two fragile X males. *Am J Med Genet* 1999; 84: 221–5.
  - 20 Gunduz MI, Gumus BH, Sekuri C. Relationship between metabolic syndrome and erectile dysfunction. *Asian J Androl* 2004; 6:355–8.
  - 21 Berkovitz GD, Wilson DP, Carpenter NJ, Brown TR, Migeon CJ. Gonadal function in men with the Martin-Bell (fragile-X) syndrome. *Am J Med Genet* 1986; 23: 227–39.
  - 22 Slegtenhorst-Eegdeman KE, de Rooij DG, Verhoef-Post M, van de Kant HJ, Bakker CE, Oostra BA, *et al.* Macroorchidism in FMR1 knockout mice is caused by increased Sertoli cell proliferation during testicular development. *Endocrinology* 1998; 139: 156–62.
  - 23 Sutherland GR. Fragile sites on human chromosomes: Demonstration of their dependence on the type of tissue culture medium. *Science* 1977; 197: 265–6.