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·*Original Article*·

Erectile dysfunction in Fragile X patients

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Abstract

Aim: To study a possible defect in spermatogenesis of Fragile X syndrome (FXS) patients. **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 (ED). Multiple examinations of the same subject at different times, and of different subjects from different provinces by different physicians, showed the same result consistently in all five subjects examined. **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 were 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. The finding of ED in Fragile X patients may help better understand the clinical spectrum and pathogenesis of the disease. *(Asian J Androl 2006 Jul; 8: 483–487)*

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

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1 Introduction

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

ticles 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 *FMR1* 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 *Drosophila* 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 (ED). The finding of ED 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 palates (subjects A and C), single palmar crease (subject B), flat feet (subject D) and strabismus (subject A).

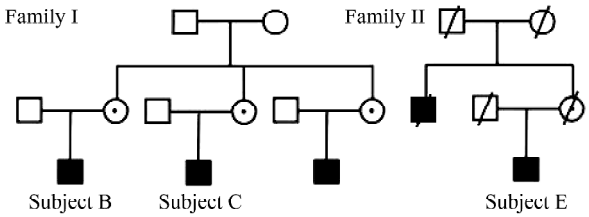

Figure 1. The pedigrees of two Fragile X syndrome (FXS) families. A square represents a man; a circle a woman; a circle with a center dot female carrier. A forward slash indicates deceased; a filled square an FXS man.

All of them showed similar behavioral symptoms including hyperactivity, hand biting (subject B showed obvious 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.

2.2 Polymerase chain reaction (PCR) 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 *FMR1*. In general, methylation is associated with a full mutation, whereas premutation and the normal allele of *FMR1* show no methylation in the 5′ UTR. The methylation sensitive PCR of the 5′ UTR of *FMR1* 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 (New England Biolabs, Beverly, MA, USA), and then used as templates for PCR with primers FRAX-g (5′-AGTGCGACCTGTCACCGCCCT-TCAGCCTTC-3′) and FRAX-h (5′-GAAACCACGT-CACGTGATCAACGCTGTTCC-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′-CTGGCACCCTATGGACACG-3′ and 5′-GTCTTCTGGGTGGCAGTGAT-3′) giving rise to a 367 bp band of glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), a house-keeping gene, was included in the PCR system as an internal control.

2.3 Sperm collection

Sperm collection was done mostly according to the laboratory manual of the World Health Organization [15]. Briefly, penis stimulation was performed by self- 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 *FMR1* 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 a house-keeping gene encoding GAPDH was positive for all samples (Figure 2). Taking the clinical profiles and molecular analyses together, we concluded that the five subjects had FXS.

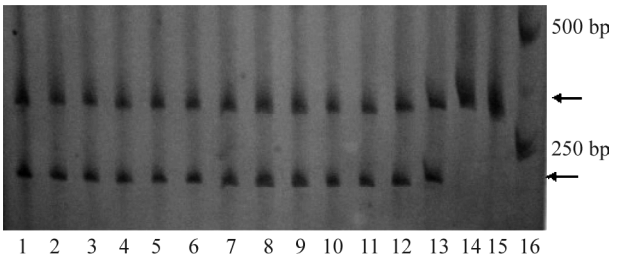

Figure 2. Polymerase chain reaction (PCR) analysis of the methylation status of the *FMR1* 5′UTR region. Lanes 1–10 were samples from subjects A–E (two adjacent lanes for each subject); lanes 11–12 from a subject previously confirmed as having Fragile X syndrome (FXS) by Southern blot; lanes 13–15 from 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 *FMR1*. All samples showed the upper control band (lane 1-15). All patients showed the *FMR1* 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).

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) in a private setting, but failed in all of them as a result of an unexpected ED. 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. ED 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 ED. 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 the case 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]. A special form of "size mosaicism" is well established that late-stage sperm cells always contain a premutation, whereas peripheryl 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 ED, as we present here. Many conditions can cause ED [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 ED in the five FXS men examined, it has been reported that an array of sex hormones in 15 FXS males and 4 other mentally retarded subjects with macroorchidism was normal [7, 21]. 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 ED 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 ED and to what extent ED is associated with macroorchidism and the absence of FMRP. Based on our experience that all five subjects examined had ED, 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.

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