European Journal of Medical Genetics 59 (2016) 459–462

Contents lists available at ScienceDirect

European Journal of Medical Genetics

journal homepage: http://www.elsevier.com/locate/ejmg

Germinal mosaicism for a deletion of the *FMR1* gene leading to fragile X syndrome

P. Jiraanont [a, b], R.J. Hagerman [c, d], G. Neri [e], M. Zollino [e], M. Murdolo [e], F. Tassone [a, d, *]

[a] Department of Biochemistry and Molecular Medicine, University of California Davis, Davis, CA, USA
[b] Research Center for Neuroscience, Institute of Molecular Biosciences, Mahidol University, Nakornpathom, Thailand
[c] Department of Pediatrics, University of California Davis, Davis, CA, USA
[d] MIND Institute, School of Medicine, University of California Davis, Davis, CA, USA
[e] Institute of Medical Genetics, Catholic University of the Sacred Heart-Agostino Gemelli University Hospital, Rome, Italy

ARTICLE INFO

Article history:
Received 9 August 2016
Accepted 9 August 2016
Available online 18 August 2016

Keywords:
Germinal mosaicism
Fragile X
FMR1 gene
Large deletions

ABSTRACT

Aberrant CGG trinucleotide amplification within the *FMR1* gene, which spans approximately 38 Kb of genomic DNA is almost always what leads to fragile X syndrome (FXS). However, deletions of part or the entire *FMR1* gene can also cause FXS. Both CGG amplification-induced silencing and deletions result in the absence of the *FMR1* gene product, FMRP. Here, we report a rare case of germinal mosaicism of a deletion encompassing approximately 300 Kb of DNA, which by removing the entire *FMR1* gene led to FXS. The male proband, carrying the deletion, presented in clinic with the typical features of FXS. His mother was analyzed by FISH on metaphase chromosomes with cosmid probe c22.3 spanning the *FMR1* locus, and she was found not to carry the deletion on 30 analyzed cells from peripheral blood lymphocytes. Prenatal examination of the mother's third pregnancy showed that the male fetus also had the same deletion as the proband. Following this prenatal diagnosis, FISH analysis in the mother was expanded to 400 metaphases from peripheral lymphocytes, and a heterozygous *FMR1* deletion was found in three. Although this result could be considered questionable from a diagnostic point of view, it indicates that the deletion is in the ovary's germinal cells.

1. Introduction

Fragile X syndrome (FXS) is the most common form of X-linked intellectual and developmental disability, occurring approximately in 1 out of 5000 males and 1 out of 4000–8000 females (Coffee et al., 2008; Maddalena et al., 2001). FXS is caused by an expansion of the CGG trinucleotide repeat within the 5'UTR of the *FMR1* gene located on chromosome Xq27.3, in correspondence with the FRAXA fragile site (Yu et al., 1991). Expansion of the CGG repeat to greater than 200 (full mutation) results in hypermethylation, aberrant heterochromatinization, and transcriptional silencing of *FMR1* leading to loss or absence of the gene product FMRP (Fu et al., 1991). Affected males with the full mutation phenotypically have moderate to severe intellectual disability, large ears, prominent jaw, joint laxity, and macroorchidism, in addition to hyperactivity, attention deficits, and autism-like behaviors (Hagerman and Hagerman, 2002).

Although FXS is commonly caused by the CGG trinucleotide repeat expansion, several reports have demonstrated that deletions encompassing the *FMR1* gene and part of it can also lead to FXS (Fan et al., 2005; Han et al., 2006; Wells, 2009). Comprehensive reviews provided by Hammond et al. (1997) and Coffee et al. (2008) have classified deletions of *FMR1* gene into two classes: small deletions (<10 kb) restricted to the 5′ end of the gene and caused by instability of the CGG repeat, and large deletions (up to 13 Mb), which are not related to CGG repeat instability and may be cytogenetically visible and often encompass adjacent genes (Coffee et al., 2008; Hammond et al., 1997). Patients carrying large deletions spanning the *FMR1* gene present with the typical clinical features of FXS due to the absence of FMRP (Gu et al., 1994; Hirst et al., 1995). However, those reported in the literature seem to have occurred *de novo* and none appears to be derived from a parental germinal mosaicism.

Germinal mosaicism or gonadal mosaicism refers to the phenomenon of phenotypically and apparently genotypically normal parents who have more than one of their children affected with an

* Corresponding author. Department of Biochemistry and Molecular Medicine, 2805 50th Street, Sacramento, CA 95817, USA.
E-mail address: ftassone@ucdavis.edu (F. Tassone).

http://dx.doi.org/10.1016/j.ejmg.2016.08.009
1769-7212/

autosomal dominant or an X-linked recessive disorder (Hall, 1988; Witkowski, 1992). In some patients, one of the parents might exhibit a somatic new mutation in a mosaic mode and transmit that gene to his or her children (Selby, 1998). Advanced molecular genetic techniques have been developed but have unveiled relatively few examples of germline mosaicism, such as Duchenne muscular dystrophy (DMD) (Wieland et al., 1991), Angelman syndrome (Kokkonen and Leisti, 2000), and Down syndrome (Delhanty, 2011).

Here we report, to our knowledge, the first documented case of germinal mosaicism for a large 300 Kb deletion spanning the entire *FMR1* gene observed in a male presenting with typical features of FXS. The mother had a second pregnancy with a normal female and a third pregnancy with a male fetus carrying the same deletion as the proband. This reproductive history is strongly suggestive of parental germinal mosaicism.

This case of germinal mosaicism demonstrates the importance and the impact of genetic counseling for reproductive decision making in order to prevent recurrence of children affected by FXS.

2. Methods

2.1. Array-CGH

It was performed on genomic DNA from uncultured peripheral blood cells at an average resolution of 35 kb (244 K kit, Agilent Technologies, Santa Clara, CA, USA), following the manufacturer's instructions. Probe alignments were referred to NCBI 37, UCSC hg19 build.

2.2. Locus-specific FISH experiments

FISH analyses were carried out on metaphase chromosomes according to standardized procedures.

2.3. Southern blot and PCR analysis

Genomic DNA of the proband, mother, and sister were isolated from peripheral blood leukocytes (3–5 ml of whole blood) using standard methods (Puregene Kit; Gentra Inc., Minneapolis, MN). PCR and Southern blot analysis were performed to determine the CGG sizing and methylation status. For Southern Blot analysis, 5–10 μg of DNA digested with EcoRI and NruI fixed on a nylon membrane was hybridized with the *FMR1* genomic probe StB12.3, labeled with Dig-11-dUTP by PCR (PCR Dig Synthesis Kit; Roche Diagnostics) following the protocol as previously described (Filipovic-Sadic et al., 2010; Tassone et al., 2008). *FMR1* and *FMR2* genes of the proband were also examined by CGH arrays (Agilent with 44,000 probes) and Multiplex Ligation-dependent Probe Amplification (MLPA) assay which revealed the presence of a deletion.

All participants signed a written consent form according to the Institutional Review Board at the University of California, Davis.

3. Results

3.1. Clinical history

The proband is a 6-1/2 year old boy who was diagnosed with FXS at approximately 3 years of age. His mother had a normal pregnancy and delivered by C-section. The proband's birth weight was 3.78 kg. He had mild problems in coordinating a suck after birth but he was eventually successfully breastfed for 3 months. His early development was mildly delayed with sitting at 9 months, crawling at 18 months, walking independently soon after 18 months. Although he said a couple of words at 2 years of age, they disappeared and his speech did not improve until speech and language therapy was initiated after he was 2 years old. Between the age of 3 and 4 years he began to put short phrases together.

His behaviors included hand-flapping, tantrums, significant anxiety, tactile defensiveness and inattention with distractibility. He has always been sensitive to loud noises and he is also perseverative in his behavior and language.

He has a history of bilateral esotropia with the right eye weaker than the left and he had intermittent patching of his left eye. He did not have a history of seizures and his EEG was normal.

On examination at the age of 6-1/2 years, his height was 111.3 cm (8th percentile), weight 19.5 kg (20th percentile), and head circumference 52 cm (50th percentile). He had a broad forehead with prominent ears with cupping of the pinnae bilaterally. He demonstrated an alternating esotropia with subtle epicanthal folds and mild drooping of the right eyelid in addition to a high arched palate. His finger joints were hyperextensible and his thumbs double-jointed. He had normal genitalia and a testicular volume of 4 ml bilaterally. His feet were flat with a mild degree of pronation.

His treatment included a low dose of sertraline beginning with 5 mg each day for his anxiety and to improve language development. He has responded well to this medication with an increase in speech production and less anxiety.

3.2. Molecular measures

At age 3 and 5/12 years he underwent array-CGH on DNA extracted from peripheral blood cells (244 K kit, Agilent Technologies, Santa Clara, CA, USA), which showed a 300 kb deletion on Xq27.3 encompassing the entire *FMR1* gene, with boundaries at 146,735,206 and 147,036,914 bp (NC_000006.12:g.146414070_146715778del; https://www.ncbi.nlm.nih.gov/clinvar/variation/242923/). These results were confirmed by FISH on metaphase chromosomes with the cosmid probe c22.3 containing the *FMR1* gene. The mother was a phenotypically, cognitively, and *FMR1* genotypically normal college graduate woman. She was also investigated by FISH with cosmid c22.3 on metaphase chromosomes from peripheral blood lymphocytes, with normal results (two copies of the *FMR1* locus on 30 analyzed cells). Molecular analysis was also performed by PCR and Southern blot, which showed no primers amplification and no hybridization with the *FMR1* specific probe in the proband, indicating the presence of a deletion of the *FMR1* gene (Fig. 1, lane 1), whereas his mother and younger sister (lane 2 and 3 respectively) showed the presence of two normal bands by PCR and normal patterns of hybridization for females (a normal methylated and a normal unmethylated band). Furthermore, a microdeletion in Xq27.3 was observed by *FMR1*-specific MLPA using probes within exon 1, 3, 4, 7, 9, 14, 15, 16, and 17.

Prenatal examination of the third pregnancy showed that the male fetus also had the same deletion as the proband. Following this prenatal diagnosis, FISH analysis in the mother was expanded to 400 metaphases from peripheral lymphocytes, and a heterozygous *FMR1* deletion was found in three.

4. Discussion

Although the CGG expansion leading to a reduction or an absence of *FMR1* gene product, FMRP, is the major cause of fragile X syndrome (FXS), several reports have revealed other mutational mechanisms, such as point mutations and deletions, either small or large, accounting for approximately 5% of the FXS cases (Myrick et al., 2014).

Small deletions (<10 Kb) are mostly found concomitant with full