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1 A nonsense mutation in *FMRI* causing fragile X syndrome

2

3 Karen Grønskov¹, Karen Brøndum-Nielsen^{1,2}, Alma Dedic¹, Helle Hjalgrim^{1,3}

4

5 1 Center for Applied Human Molecular Genetics, 2 Genetic counseling clinic, The

6 Kennedy Center, Gl. Landevej 7, 2600 Glostrup, Denmark. Phone: +45 43260100. Fax:

7 +45 43431130.

8 3 Danish Epilepsy Centre, Kolonivej 7, 1, 4293 Dianalund, Denmark. Phone: +45

9 58271221. Fax: +45 58271060

10

11 Corresponding author: Karen Grønskov kag@kennedy.dk

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13 Running title: *FMRI* nonsense mutation

1 ABSTRACT

2 Fragile X syndrome is a common cause of inherited intellectual disability. It is caused
3 by lack of the *FMRI* gene product FMRP. The most frequent cause is an expansion of a
4 CGG repeat located in the 5' UTR of *FMRI*. Alleles with 200 or more repeats become
5 hypermethylated and transcriptionally silent. Only few patients with intragenic point
6 mutations in *FMRI* have been reported and to-day routine analysis of patients referred
7 for fragile X syndrome includes solely analysis for repeat expansion and methylation
8 status. We identified a substitution in exon 2 of *FMRI*, c.80C>A, causing a nonsense
9 mutation p.Ser27X, in a patient with classical clinical symptoms of fragile X syndrome.
10 The mother who carried the mutation in heterozygous form presented with mild
11 intellectual impairment. We conclude that further studies including Western blot and
12 DNA sequence analysis of the *FMRI* gene should be performed in patients with typical
13 symptoms of fragile X syndrome, in whom no CGG repeat expansion is detected.

14

15 KEYWORDS

16 Fragile X syndrome, nonsense mutation

17

18 INTRODUCTION

19 Fragile X syndrome is a frequent form of inherited intellectual disability affecting
20 approximately 1 in 4000 males (reviewed by Chonchaiya et al.¹). In the vast majority of
21 patients the syndrome is caused by an expansion of a CGG repeat located in the 5'UTR
22 of the *FMRI* gene localized to Xq27.3. The gene was cloned in 1991 and comprises 17
23 coding exons. The protein, FMRP, is an RNA binding protein of 632 amino acids.²
24 Only four point mutations in *FMRI* have been reported: a missense mutation

1 p.Ile304Asn, a 1-bp deletion c.373delA in exon5 resulting in a frameshift and premature
2 truncation of the protein, a 2-bp change g.23714GG>TA spanning the intron/exon
3 boundary of exon 2, and a missense mutation p.Arg138Gln.³⁻⁵ Deletions of the CGG
4 repeat and flanking sequences have been reported several times.⁶

5

6 MATERIALS AND METHODS

7 *Patient*

8 The patient, a 35 year old mentally retarded male, was referred to a tertiary epilepsy
9 centre due to difficulties in treatment of epilepsy with absences, generalised tonic-clonic
10 seizures and myoclonias. As a further diagnostic evaluation he was tested for fragile X
11 syndrome. Phenotypically he had classical fragile X syndrome with elongated face,
12 high, broad forehead, low set large ears, prognathia and enlarged testes. Neurological
13 examination showed hypotonia and hypermobility, with hyperextensible joints. He had
14 no active language except a few repeated words used out of context, and showed autistic
15 features with little eye contact, discomfort upon physical contact, perseveration and
16 limited interests. He was described by his mother as showing late motor development in
17 childhood and having need for speech therapy, and special day-care and schooling.
18 Epilepsy debuted at age 4 years.

19 The mother presented with mild to moderate intellectual disability. She reported special
20 education needs and carried out sheltered work. She was married and capable of
21 maintaining a household. Physical examination showed a relatively unremarkable
22 phenotype, but her behaviour included hypermotor activity and many automatisms. She
23 was capable of a simple conversation.

24 *Molecular biology procedures*

1 Genomic DNA was extracted from peripheral blood lymphocytes (EDTA stabilized)
2 using standard procedures.

3 Lymphobalstoid cell lines were established from the patient and his mother using
4 Epstein Barr virus transformation.

5 Southern blot analysis was performed as described.⁷ Briefly, 9 µg of high molecular
6 genomic DNA was digested with Pst1 and 6 µg DNA was digested with EcoR1 plus
7 EagI and size separated on an agarose gel. Standard blotting technique was performed to
8 transfer DNA to nitrocellulose filter, which was hybridized with radioactive labeled
9 pPX6 probe. After washing the filter was analyzed using a Cyclone from Perkin Elmer
10 (Waltham, Massachusetts, USA).

11 PCR analysis of the *FMRI* CGG repeat was performed using the fragile X kit from
12 Abbott Molecular (Illinois, USA) following the manufacturer's instructions.

13 Western blot analysis was performed as described.⁷

14 Mutational analysis of *FMRI* was performed by direct DNA sequencing of PCR
15 products of coding exons and at least 20 bp of flanking sequences. Primers and PCR
16 conditions are available upon request. Sequencing was performed using BigDye v3.1
17 terminator chemistry, and an ABI3130XL genetic analyzer and using the Seqscape
18 program for analysis (Applied Biosystems, Foster City, CA, USA).

19 X-inactivation analysis was performed using the polymorphic CAG repeat in the
20 androgen receptor locus as described.⁷

21

22 RESULTS

23 The patient and his mother are shown in figure 1.

1 Southern blot analyses of FRAXA (EcoRI+EagI and PstI digestion respectively)
2 showed band patterns corresponding to a repeat number in the normal range (Figure 2A
3 and Figure 2B). PCR analysis showed an allele size corresponding to 29 repeats.
4 However, based on the classic clinical fragile X appearance we performed a Western
5 blot using an anti-FMRP antibody, which showed that no FMRP was expressed (Figure
6 2C). We also performed Southern blot analysis and PCR of FRAXA of the mother of the
7 patient; this showed a normal band pattern and repeat number of 29 and 33 with no
8 indication of a full mutation. Subsequent DNA sequencing revealed a sequence
9 variation in exon 2 of *FMRI* c.80C>A resulting in a nonsense mutation p.Ser27X.
10 Verification of the sequence variation was performed on a second PCR product, and the
11 variation was seen in both directions. The mother was found to be heterozygous for the
12 mutation. We investigated X-inactivation pattern using a polymorphic CAG repeat in
13 the androgen receptor locus and found an equal distribution of active and inactive X-
14 chromosomes in blood (data not shown)..

15

16 DISCUSSION

17 We report a nonsense mutation in *FMRI* in a patient with classic fragile X syndrome
18 and his carrier mother with mild intellectual impairment. The fragile X syndrome can be
19 considered semi-dominant with manifestations in female carriers of a full mutation
20 depending on the proportion of active versus inactive X chromosomes carrying the
21 mutation. Older studies showed that phenotypical normal females have a skewed X
22 inactivation pattern in favor of the inactive X carrying the mutation.⁸ Since the X
23 inactivation in the mother showed an equal distribution, some degree of intellectual

1 impairment would be expected. However, this study was performed in blood and
2 therefore is an approximation since we do not know the pattern in brain.

3 Surprisingly few point mutations have been found in the *FMRI* gene. Two of these are
4 truncating mutations in the N-terminal half of the gene and supposedly lead to nonsense
5 mediated decay of the mRNA. These two mutations caused classical fragile X
6 syndrome.⁵ Another patient had a missense mutation in one of the KH domains and had
7 a more severe phenotype.⁴ This could be due to a gain-of-function effect; however, in
8 this family a glucokinase defect was also present which might have contributed to the
9 phenotype. The observed low frequency of point mutations in *FMRI* might be because
10 *FMRI* is not routinely screened for point mutations, but only investigated for the
11 common CGG expansion; another reason could be that point mutations in this gene give
12 atypical or even lethal phenotypes. More than 1100 patients have been screened for
13 point mutations in *FMRI*,^{3,9-15} however only one missense mutation of questionable
14 pathogeneity was identified.³ The individuals investigated in these studies represent a
15 very heterogenous group of patients with developmental delay, indicating that *FMRI*
16 mutations are not a common cause for mental impairment. To our knowledge, a
17 mutational screening of *FMRI* has not been performed in a large cohort of patients with
18 typical fragile X syndrome negative for repeat expansion. Therefore, the frequency of
19 point mutations in *FMRI* in classical fragile X syndrome is unknown. The patient
20 presented in this study shows a typical fragile X phenotype, and his mother shows a
21 phenotype comparable to a female with a full mutation. We therefore suggest that in
22 patients with clinical fragile X syndrome and no CGG expansion, expanded molecular
23 diagnosis should be considered including Western blot analysis and DNA sequencing.

24

1 CONFLICT OF INTEREST

2 The authors declare no conflict of interest.

3

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23
24
25

1 TITLES AND LEGENDS TO FIGURES

2

3 Figure 1

4 Facial appearance of the a) patient and b) his mother. Note the elongated face, high
5 broad forehead and prognathia in patient (a).

6

7 Figure 2

8 **A.** Southern blot analysis using EcoRI+EagI digestion and **B.** Southern blot analysis
9 using PstI digestion. P: patient; M: mother; FM ♀: full mutated female; PM ♀:
10 premutated female; FM ♂: full mutated male; PM ♂: premutated male; N ♂ normal
11 male; N ♀: normal female. **C.** Western blot analysis using an anti-FMRP antibody.
12 Patient (P); FM ♂: full mutated male; N ♂ normal male.

a)



b)



Figure 1

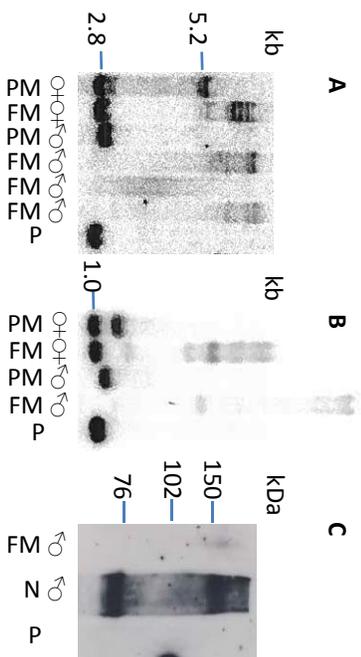


Figure 2