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SHORT REPORT

A nonsense mutation in *FMR1* causing fragile X syndrome

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Fragile X syndrome is a common cause of inherited intellectual disability. It is caused by lack of the *FMR1* gene product FMRP. The most frequent cause is the expansion of a CGG repeat located in the 5′UTR of *FMR1*. Alleles with 200 or more repeats become hypermethylated and transcriptionally silent. Only few patients with intragenic point mutations in *FMR1* have been reported and, currently, routine analysis of patients referred for fragile X syndrome includes solely analysis for repeat expansion and methylation status. We identified a substitution in exon 2 of *FMR1*, c.80C>A, causing a nonsense mutation p.Ser27X, in a patient with classical clinical symptoms of fragile X syndrome. The mother who carried the mutation in heterozygous form presented with mild intellectual impairment. We conclude that further studies including western blot and DNA sequence analysis of the *FMR1* gene should be performed in patients with typical symptoms of fragile X syndrome in whom no CGG repeat expansion is detected.
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INTRODUCTION

Fragile X syndrome is a frequent form of inherited intellectual disability affecting approximately 1 in 4000 males (reviewed by Chonchaiya *et al.*[1]). In the vast majority of patients the syndrome is caused by expansion of a CGG repeat located in the 5′UTR of the *FMR1* gene localized to Xq27.3. The gene was cloned in 1991 and comprises 17 coding exons. The protein, FMRP, is an RNA-binding protein with 632 amino acids.[2] Only four point mutations in *FMR1* have been reported: a missense mutation p.Ile304Asn, a 1-bp deletion c.373delA in exon 5 resulting in a frameshift and premature truncation of the protein, a 2-bp change g.23714GG>TA spanning the intron/exon boundary of exon 2, and a missense mutation p.Arg138Gln.[3–5] Deletions of the CGG repeat and flanking sequences have been reported several times.[6]

PATIENTS AND METHODS

Patient

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

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

Molecular biology procedures

Genomic DNA was extracted from peripheral blood lymphocytes (EDTA stabilized) using standard procedures. Lymphoblastoid cell lines were established from the patient and his mother using Epstein–Barr virus transformation.

Southern blot analysis was performed as described.[7] Briefly, 9 μg of high molecular genomic DNA was digested with *Pst*1, and 6 μg DNA was digested with *Eco*R1 plus *Eag*I and size-separated on an agarose gel. Standard blotting technique was used to transfer DNA to a nitrocellulose filter, which was hybridized with radioactive labelled pPX6 probe. After washing, the filter was analysed using a Cyclone from Perkin-Elmer (Waltham, MA, USA).

PCR analysis of the *FMR1* CGG repeat was performed using the fragile X kit from Abbott Molecular (IL, USA) following the manufacturer's instructions. Western blot analysis was performed as described.[7]

Mutational analysis of *FMR1* was performed by direct DNA sequencing of PCR products of coding exons and at least 20 bp of flanking sequences. Primers and PCR conditions are available upon request. Sequencing was performed using BigDye v3.1 terminator chemistry and an ABI3130XL genetic analyzer and using the Seqscape program for analysis (Applied Biosystems, Foster City, CA, USA). X-inactivation analysis was performed using the polymorphic CAG repeat in the androgen receptor locus as described.[7]

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RESULTS

The patient and his mother are shown in Figure 1.

Southern blot analyses of FRAXA (*Eco*RI+*Eag*I and *Pst*I digestion, respectively) showed band patterns corresponding to a repeat number in the normal range (Figures 2a and b). PCR analysis showed an allele size corresponding to 29 repeats. However, based on the classic clinical fragile X appearance, we performed a western blot using an anti-FMRP antibody, which showed that no FMRP was expressed (Figure 2c and Supplementary Figure 1). We also performed Southern blot analysis and PCR of FRAXA of the mother of the patient; this showed a normal band pattern and repeat numbers of 29 and 33, with no indication of a full mutation (data not shown). Subsequent DNA sequencing revealed a sequence variation in exon 2 of *FMR1* c.80C>A resulting in a nonsense mutation p.Ser27X (Supplementary Figure 2). Verification of the sequence variation was performed on a second PCR product, and the variation was seen in both directions. The mother was found to be heterozygous for the mutation. We investigated X-inactivation pattern using a polymorphic CAG repeat in the androgen receptor locus and found an equal distribution of active and inactive X-chromosomes in blood (data not shown).

DISCUSSION

We report a nonsense mutation in *FMR1* in a patient with classic fragile X syndrome and his carrier mother with mild intellectual impairment. The fragile X syndrome can be considered semi-dominant, with manifestations of a full mutation in female carriers, depending on the proportion of active versus inactive X chromosomes carrying the mutation. Earlier studies have shown that phenotypical normal females have a skewed X inactivation pattern in favour of the inactive X carrying the mutation.[8] As the X inactivation in the mother showed an equal distribution, some degree of intellectual impairment would be expected. However, this study was performed in blood and therefore is an approximation, as we do not know the pattern in brain.

Surprisingly, few point mutations have been found in the *FMR1* gene. Two of these are truncating mutations in the N-terminal half of the gene and supposedly lead to nonsense-mediated decay of the mRNA. These two mutations caused classical fragile X syndrome.[5] Another patient had a missense mutation in one of the KH domains and had a more severe phenotype.[4] This could be due to a gain-of-function effect; however, in this family X-linked glycogenosis was also present, which might have contributed to the phenotype. The observed low frequency of point mutations in *FMR1* might be because *FMR1* is not routinely screened for point mutations, but is only investigated for the common CGG expansion; another reason could be that point mutations in this gene give atypical or even lethal phenotypes. More than 1100 patients have been screened for point mutations in *FMR1*;[3,9–15] however, only one missense mutation of questionable pathogeneity was identified.[3] The individuals investigated in these studies represent a very heterogenous group of patients with developmental delay, indicating that *FMR1* mutations are not a common cause for mental impairment. To our knowledge, a mutational screening of *FMR1* has not been performed in a large cohort of patients with typical fragile X syndrome negative for repeat expansion. Therefore, the frequency of point mutations in *FMR1* in classical fragile X syndrome is unknown. The patient presented in this study shows a typical fragile X phenotype, and his mother shows a phenotype comparable to a female with a full mutation. We therefore suggest that in patients with clinical fragile X syndrome and no CGG expansion expanded molecular diagnosis should be considered, including western blot analysis and DNA sequencing.

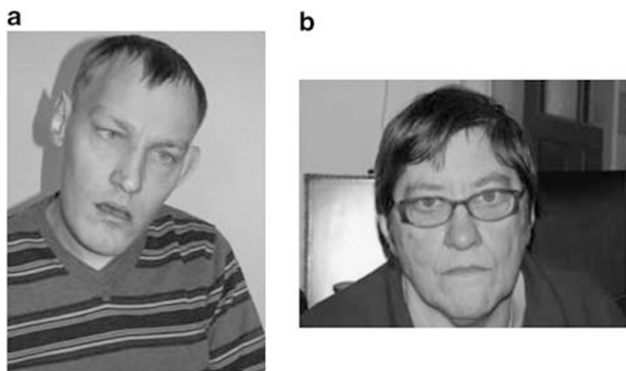

Figure 1 Facial appearance of (**a**) the patient and (**b**) his mother. Note the elongated face, high broad forehead and prognathia in the patient (**a**).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

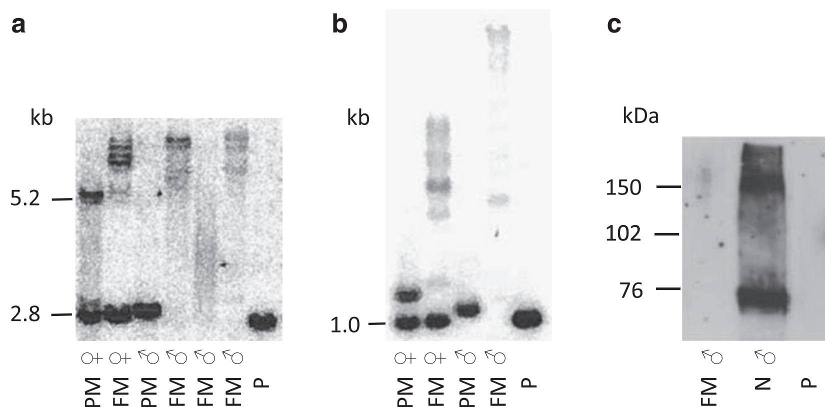

Figure 2 Southern blot analysis using (**a**) *Eco*RI+*Eag*I digestion and (**b**) *Pst*I digestion. P, patient; M, mother; FM ♀, full mutated female; PM ♀, premutated female; FM ♂, full mutated male; PM ♂, premutated male; N ♂, normal male; N ♀, normal female. (**c**) Western blot analysis using an anti-FMRP antibody. P, patient; FM ♂, full mutated male; N ♂ normal male.

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Supplementary Information accompanies the paper on European Journal of Human Genetics website (http://www.nature.com/ejhg)