



## Original Article

## Molecular Screening of Fragile X Syndrome in Children with Mental Retardation in Hualien

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### Article info

#### Article history:

Received: March 28, 2008

Revised: June 9, 2008

Accepted: June 24, 2008

#### Keywords:

*FMR1* gene

Fragile X syndrome

Mental retardation

PCR

### Abstract

**Objective:** Fragile X syndrome is the most common inherited form of X-linked mental retardation (XLMR). The aim of this study was to screen for and identify fragile X syndrome-affected individuals using DNA-based molecular approaches at a special education school for the mentally retarded in Hualien, Taiwan.

**Materials and Methods:** Genomic DNA extracted from 217 individuals with mental retardation, including 148 males and 69 females, was analyzed using polymerase chain reaction, DNA gel electrophoresis and Southern blot analysis. Individuals with the full mutation or premutation were determined according to the CGG repeat size in the 5'-untranslated region in the *FMR1* gene on chromosome Xq27.3.

**Results:** A total of 217 individuals with mental retardation were screened, and three (1.38%) were identified as having fragile X full mutation. Two of the 148 males (1.35%) and one of the 69 females (1.44%) had this mutation. The incidence is very close to that in previous reports in Western Taiwan. Female carriers of fragile X syndrome were not found in this study.

**Conclusion:** We established a molecular screening approach and report the incidence of fragile X syndrome in a mentally retarded population in Eastern Taiwan. The molecular study and genetic counseling for other family members will continue in the future. (*Tzu Chi Med J* 2008;20(4):309–313)

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## 1. Introduction

Fragile X syndrome (OMIM: 309550), existence of cytogenetic fragile site on Xq27.3, is the most common form of X-linked mental retardation (XLMR) (1). It has been noted that fragile X syndrome is an inherited

mental retardation disorder, and is the second most identifiable cause of mental retardation after Down syndrome. The frequency of fragile X syndrome in males is estimated to be 1 in 4000 (2,3). Affected males with fragile X syndrome usually have an IQ range of 20–70. Other clinical features include a long narrow

face, prominent ears and jaw, macroorchidism, flat feet and autistic behavior (4–6).

Fragile X syndrome is caused by a CGG-repeated expansion within the 5' untranslated region (5'-UTR) of the fragile X mental retardation 1 (*FMR1*) gene on the q arm of the X chromosome (7). The number of trinucleotide repeats in the population is classified as normal, premutation, and full mutation. The normal CGG repeat size is polymorphic below 54. Asymptomatic fragile X carriers have a repeat size ranging from 55 to 200, which is termed premutation. When the trinucleotide expansion length exceeds 200 repeats in affected individuals, it is termed full mutation of the *FMR1* gene (3,8,9). It is now known that in CGG repeats expanded to full mutation length, hypermethylation of the CpG islands in the promoter region of the *FMR1* gene occurs and gene expression is silenced. The transcriptional inactivation of the *FMR1* gene leads to absence of protein product and development of the phenotype of fragile X syndrome (4,10). Identification of the size of the CGG repeat is the basis of molecular testing for fragile X syndrome.

To date, treatment for fragile X syndrome is not yet available. However, this disorder can be diagnosed by molecular analysis of the *FMR1* gene mutation. Polymerase chain reaction (PCR)-based DNA analysis of the *FMR1* gene to detect carriers and the full mutation is well established (11,12). In Caucasian populations, approximately 2–6% of males and 2–4% of females with nonspecific retardation carry the full fragile X mutation (13,14). The full mutation rate determined by molecular testing in the mentally retarded population in China is approximately 2.8%, similar to that in Caucasian populations (15). The incidence detected by DNA-based screening of the full mutation for nonspecific mental retardation populations is approximately 1–2% in Western Taiwan (11,16). However, the incidence of fragile X syndrome in mentally retarded populations in Eastern Taiwan is not clear.

The aim of this study was to screen for and identify individuals with fragile X syndrome using DNA-based testing methods at a special education school for the mentally retarded in Hualien, Taiwan.

## 2. Materials and methods

### 2.1. Subjects

A total of 217 individuals with mental retardation, including 148 males (68.2%) and 69 females (31.8%), from the *National Hualien Special Education School for the Mentally Retarded* were analyzed for molecular identification of fragile X syndrome. The ages of the subjects ranged between 6 and 22 years old as shown in Table 1. This study was approved by the Institutional Review Board of Buddhist Tzu Chi Medical Center

**Table 1 — Molecular testing for fragile X syndrome in subjects with mental retardation from a special education school**

	Males (n=148)	Females (n=69)	Total (n=217)
Age range, yr	6–21	6–22	6–22
Molecular testing by PCR	127	0	127
Molecular testing by Southern blot analysis	25	69	92
Full mutation of <i>FMR1</i> gene	2 (1.35%)	1 (1.44%)	3 (1.38%)
Premutation of <i>FMR1</i> gene	0	0	0

PCR=polymerase chain reaction.

(IRB092-22) and written informed consents were provided by the parents or guardians before sampling of blood for DNA analysis.

### 2.2. Genomic DNA preparation and PCR

Genomic DNA was isolated from blood samples using a commercial kit (Gentra Systems Inc., Big Lake, MN, USA) according to the manufacturer's instructions. The CGG repeat length of *FMR1* 5'-UTR was analyzed by PCR following polyacrylamide gel electrophoresis separation.

PCR was performed as described previously (11), but with modifications. Briefly, the *FMR1* DNA fragment was amplified in a volume of 30  $\mu$ L using genomic DNA as template, 1X PCR buffer, 50  $\mu$ M deoxynucleotide triphosphate (dNTP), 100 nM of forward and reverse primers, 0.5 M betaine, 10% glycerol, 10% dimethyl sulfoxide, and 0.6 U SuperTherm DNA polymerase or FastStart Taq DNA polymerase (Roche Applied Science, Penzberg, Germany). PCR was carried out for 35 cycles in a thermal cycler with 30 seconds of denaturing at 95°C, 30 seconds of annealing at 64°C, a 1-minute extension at 72°C, and a 10-minute final extension step to complete the reaction at 72°C. The PCR products from male genomic DNA were analyzed by 6% polyacrylamide gel electrophoresis followed by ethidium bromide staining and ultraviolet detection.

### 2.3. Southern blot analysis

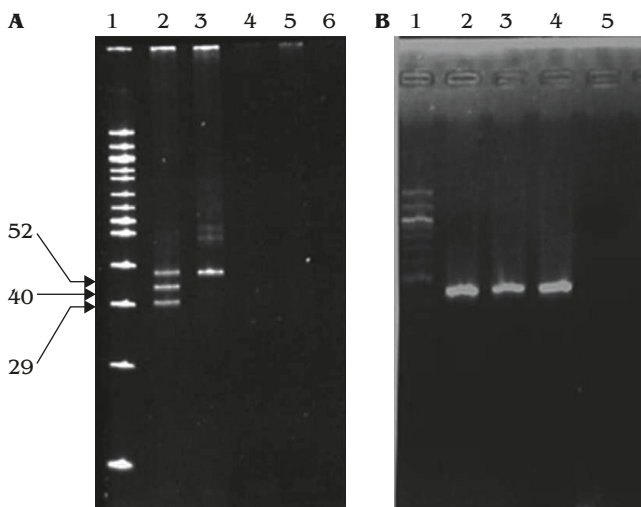
Southern blot analysis to verify *FMR1* gene methylation was performed using protocols with a non-radioactive chemiluminescent assay as described in a previous report (11). Genomic DNA was digested with restriction enzymes, *EcoRI* and *NruI*, and separated by 0.8% agarose gel electrophoresis. DNA was transferred onto the nylon membrane and hybridized with a digoxigenin-labeled probe. After 24 hours of hybridization, the blot was washed with 0.5  $\times$  SSC containing 0.1% SDS at 60°C, and the DNA band was detected by incubating

the blot with the chemiluminescent reagent (Roche Applied Science) followed by radiographic film exposure for 4 hours.

### 3. Results

#### 3.1. Molecular screening of males for *FMR1* gene mutation by PCR

Table 1 summarizes the results of molecular testing for fragile X syndrome in this study. A total of 148 males with mental retardation from a special education school were analyzed for fragile X syndrome. PCR was performed clearly in 127 out of 148 males, and 21 individuals had ambiguous results on the molecular testing. The normal CGG repeat size in the *FMR1* gene was below 54 and could be detected in the range of 200–300 bp of PCR products followed by 6% polyacrylamide gel electrophoresis (Fig. 1A, lanes 2 and 3). The results were normal in 125 out of 127 males with PCR followed by DNA gel electrophoresis in this study. DNA amplification could not be performed well by DNA polymerase for full mutations of the *FMR1* gene because the repeating length



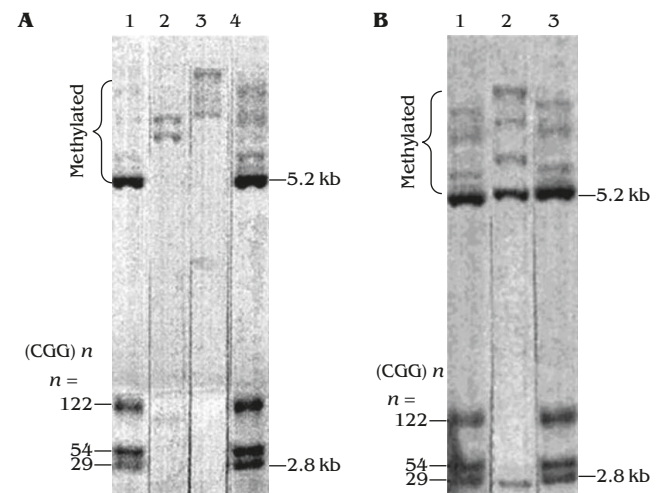
**Fig. 1** — Examples of polymerase chain reaction (PCR) amplification results of the *FMR1* gene. PCR DNA products analyzed by 6% polyacrylamide gel electrophoresis. (A) PCR products of the *FMR1* gene. Lane 1=100-bp DNA marker; lane 2=CGG-repeated DNA control marker; lane 3=52-CGG-repeat positive control; lane 6=negative control. Arrows indicate the size of CGG-repeated DNA control marker numbers with 29- (lower), 40- (middle), and 52-CGG (upper) repeats. The PCR products of two affected male DNA samples (full mutation) are undetectable in lanes 4 and 5. (B) PCR products of the dystrophin gene. Lane 1=DNA marker; lanes 2 and 3=DNA samples from the two affected males (full mutation); lane 4=normal control; lane 5=negative control.

was more than 200 CGG repeats. Fig. 1 shows that undetectable PCR products in two out of 127 males may have been affected by full mutation of the *FMR1* gene (Fig. 1A, lanes 4 and 5, with undetectable DNA bands in the range of normal CGG repeat size).

To rule out unqualified DNA templates leading to undetectable DNA products during PCR, another PCR of the dystrophin gene was performed as a reaction control, and the dystrophin DNA could be detected on the gel, as expected, in the two affected males (Fig. 1B, lanes 2 and 3, compared with Fig. 1A, lanes 4 and 5). The results show that the two boys had fragile X syndrome.

#### 3.2. Southern blot analysis of affected males and all females for verifying full mutation of *FMR1* gene

To verify the mutation type and carriers of fragile X syndrome, all 69 females with mental retardation and 23 males, including 21 males with ambiguous diagnosis on PCR analysis and two affected individuals with undetectable PCR products as described previously (Fig. 1A), were analyzed using the Southern blot technique (Table 1). In unaffected individuals with fragile X full mutation, one single hybridizing band of 2.8 kb with a normal range of CGG repeats was detected in males and one further band of 5.2 kb was detected in females due to X chromosome inactivation (Fig. 2A, lanes 1 and 4). The affected individual with full mutation presented smear bands larger than



**Fig. 2** — Southern blot analysis of the *FMR1* gene from three affected individuals. (A) Hybridization result from the two affected boys. Lanes 1 and 4=normal controls; lanes 2 and 3=full mutation. (B) Hybridization result from the one affected girl. Lanes 1 and 3=normal controls; lane 2=full mutation.

5.9kb—due to hypermethylation and repeat expansion of the *FMR1* gene. Fig. 2A shows that only the two affected males identified by PCR testing showed hypermethylated bands on Southern hybridization analysis (Fig. 2A, lanes 2 and 3). This result confirmed the PCR data and indicated that two of these 148 males (1.35%) with mental retardation had full mutation of the *FMR1* gene.

One of the 69 females (1.44%) tested was identified as having the full mutation. Fig. 2B shows that the affected female has one normal allele (lane 2, 2.8 kb and 5.2 kb bands) and one methylated allele with smear bands of full mutation (lane 2, bands >5.2 kb). A total of 217 individuals with mental retardation were screened with PCR and Southern blot analysis, and three (1.38%), including two males and one female, were identified to have fragile X full mutation.

### 3.3. The families of individuals with fragile X full mutation

Genetic counseling of these affected families was performed after verification of the full mutation of the *FMR1* gene. The three individuals with fragile X syndrome belonged to two different families. Two affected individuals with fragile X full mutation in this screening, one boy and girl, were siblings. Another brother with fragile X syndrome was also found in the same family. The other affected male belonged to a different family but had another fragile X sister. The mutated *FMR1* gene was inherited from the mothers in all the affected individuals.

## 4. Discussion

In the present study, 217 mentally retarded children in Hualien were screened by molecular testing methods and three (1.38%) were identified to have full mutation of the *FMR1* gene. The frequency of fragile X syndrome in mentally retarded children in Hualien in Eastern Taiwan was 1.38% in this study, very close to that in previous reports of Western Taiwan (16).

It has been reported that the prevalence of fragile X syndrome in the Caucasian mentally retarded population from molecular screening is 2.6–8.7% in males and 2.9–5.4% in females. In Asian populations, molecular testing in Japanese and Chinese mentally retarded subjects gave prevalences of 0.8–2.7% and 2.8–6.8%, respectively (14,15,17). In Taiwan, molecular screening of children with mental retardation gave prevalences ranging from 1.5% to 2.5% (11,16,18). The incidence in this study is lower than that in China (11,15,19).

No individual with premutation of the *FMR1* gene was found in our study. It has been reported that the

frequency of fragile X syndrome in female carriers is very low in the Taiwanese population (18,20,21). The frequency of female carriers is high in Israel (1 in 113), and ranges from 1 in 186 to 1 in 259 in the United States, Finland and Canada (22–24). These studies indicate that mutation of the *FMR1* gene in Taiwanese populations may not be as common as in Caucasians.

In this study, the affected individuals belonged to two different families and appropriate genetic counseling was provided after molecular detection of fragile X syndrome. To date, no treatment is available for this disorder, but it can be prevented by genetic counseling and prenatal diagnosis. Diagnosis of fragile X syndrome among mentally retarded children by DNA-based screening methods is a practical approach. Early diagnosis, appropriate rehabilitation programs, carrier detection, genetic counseling and education are important issues for managing fragile X syndrome, especially in Eastern Taiwan.

In conclusion, we established a molecular diagnosis approach to screen mentally retarded children in Hualien for fragile X syndrome and reported the incidence of full mutation of the *FMR1* gene among these children. The continuing education of health supervisors in schools for the mentally handicapped and follow-up of families with appropriate genetic counseling will continue in the future.

## Acknowledgments

This work was supported by a research grant (TCRD92-13) from Buddhist Tzu Chi General Hospital, Hualien, and a grant (no. 93-94) from the Department of Health, Taiwan.

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