Molecular screening of FMR1 mutation among autism patients in China

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Background: **Fragile X syndrome (FXS) is the most common inherited form of mental retardation, affecting approximately 1 in 1250 males and 1 in 2500 females, with estimated premutation carriers of 1/600 in males and 1/300 in females. It is resulted from an unstable expansion of triplet CGG repeats at promoter region of the FMR1 gene, which consequently silences FMR1 gene expression. Our earlier study has determined that FXS accounts for 3.2% of the Chinese mental retarded population. Recently, FMR1 mutation has been found in autism patients. In this report, we present a pilot study of molecular screening of FMR1 mutation in a subset of Chinese autism patients.**

Methods: **A total of 235 DNA samples, including 185 samples from autism patients (174 males and 11 females) and 50 samples from mental retardation (MR) patients (36 males and 14 females), which had been banked earlier, were included. PCR based approach was employed for analyzing CGG repeat size. PCR products were detected on 6% denaturated PAGE electrophoresis and detected with silver staining.**

Results: **Among 235 samples screened, 226 (49 MR and 177 autism) samples showed normal patterns. No normal pattern could be detected among the remaining 9 samples including 1 MR and 8 autism cases.**

Conclusions: **We have developed a simple, easy, rapid and economical PCR-based molecular screening approach for detecting FMR1 CGG repeats. Using this approach, we have detected 9 subjects from our previously banked MR and autism DNA samples. We suggest the use of this approach in clinical practice for screening FXS high-risk population among pregnant women and MR children whose genetic etiology is yet unknown.**

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Key words: **fragile X syndrome; FMR1; autism; Chinese; molecular screening**

Introduction

Fragile X syndrome (FXS) is the most prevalent Mendelian inherited disease with mental retardation (MR).[1] The incidence of FXS is 1/1250 in males and 1/2500 in females, and an estimated prevalence of premutation carriers is about 1/600 in males and 1/300 in females.[2] It is characterized by moderate to severe mental retardation (IQ=10-60), long face, high palatal arch, low set ears, the reduced capability of learning, introvert characteristics, autism, and ataxia.[3] The incidence of MR in China is about 3%-8%,[4] which consists of 2.8%-3.2% fragile X syndrome.[5] Autism is correlated with Fragile X syndrome. About 2%-6% autism patients are due to FXS, and 3% FXS patients are shown to have autism.[6] The incidence of autism in China is about 0.5%.

FXS is caused by unstable expansion of a polymorphic triplet CGG repeats, at the 5' untranslated region of the FMR1 gene located at Xq27.3.[3] The CGG is characterized by 5-55 repeats in normal X chromosomes, 60-200 repeats in premutation carriers who demonstrate mild FXS clinical features or no clinical symptoms, and >200 repeats in full mutation. The range of normal and premutation CGG repeats does not affect the normal expression of FMR1.[7] In females, premutation carriers may result in sterility because the function of ovary will be decreased in advance.[8] The full mutation of CGG repeat may result in hypermethylation in the island of CpG, silence FMR1 gene expression without transcript, and lead to

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the absence of FMRP. The FMR1 encoded protein FM is an RNA-binding protein.[3]

Methods

DNA samples

A total of 235 DNA samples, which had been banked in our laboratory for other research projects, were studied. Among these DNA samples, 50 were clinically diagnosed with mental retardation (36 males and 14 females) and 185 with autism (174 males and 11 females).

PCR amplification

Genomic DNA (20-50 ng) was amplified in 25 μl reaction containing 12.5 μl 2 × GC buffer, 1 U Taq polymerase (Takara), 2.5 μl dNTPs (2 mmol) with the primers of 5'- GGA ACA GCG TTG ATC ACG TGA CGT GGT TTC -3' and 5'- GGG GCC TGC CCT AGA GCC AAG TAC CTT GT-3'.[9] The cycles were initiated with 5-minute denaturation at 95ºC, then 35 cycles at 65ºC for 1 minute, 72ºC for 2 minutes, using an ABI9800 thermocycler. The final extension was 10 minutes at 72ºC.

CGG analysis

The products of PCR of all the samples were isolated by 6% denatured PAGE electrophoresis. The voltage of electrophoresis was 600 V, and the time of electrophoresis was 4 hours. After electrophoresis, the gel was fixed in fixation solution (ethanol, glacial acetic acid) for 10 minutes, stained in 0.2% silver nitrate fluid for 15 minutes, then colored in coloration fluid (10 mol NaOH, 30% formaldehyde) until the band of PCR product became distinct.

Results

A single band of PCR product in the normal male was detected with a size between 355-505 bp on 6% denatured PAGE electrophoresis (lane 2) (Fig.). In the normal female, two bands (lanes 9 and 10) of PCR product were detected. The premutation carrier had one band between 505 and 940 bp (not shown). In the positive control FXS patient (lane 3), no band was detected by 6% denatured PAGE electrophoresis because of the large CGG repeats that could not be amplified with the current PCR condition.

In this study, the samples of 49 MR and 177 autism DNA were assessed to have CGG repeats of the FMR1 gene in normal range. Nine DNA samples, one from MR (Fig., lane 4) and eight from autism patients, showed no visible DNA band on PAGE gel. All analyses were repeated for three times.

Discussion

FXS has been recognized as the most common inherited MR, and its prevalence in the Chinese MR population is similar to that in the Caucasian.[5] A simple PCR method for detecting the CGG repeat size could be the initial choice for screening and exclusion of non-FXS MR. Therefore, we have developed a simple and rapid method for PCR-based analysis. With this method, any normal range CGG repeats would fit into the PCR size of less than 505 bp. If a single band or two bands of PCR product which are <505 bp can be detected in male or female patients respectively, FXS may be excluded. Premutation with CGG repeats of 50-200 should be between 505 bp and 904 bp and full mutation should be >904 bp. This large fragment with >95% of CG content may not be easily amplified with the primers used in this protocol. To amplify the high CG% fragment at the FMR1 promoter region, the primers should be closer to CGG repeats.[10] Even though, the PCR signal is still weak and there are lots of nonspecific amplifications. To obtain the specific PCR product of FMR1 CGG repeats, an oligo-(CGG)5 probe on Southern blotting may help to amplify the specific signals.[10,11]

When the PAGE does not present a normal size

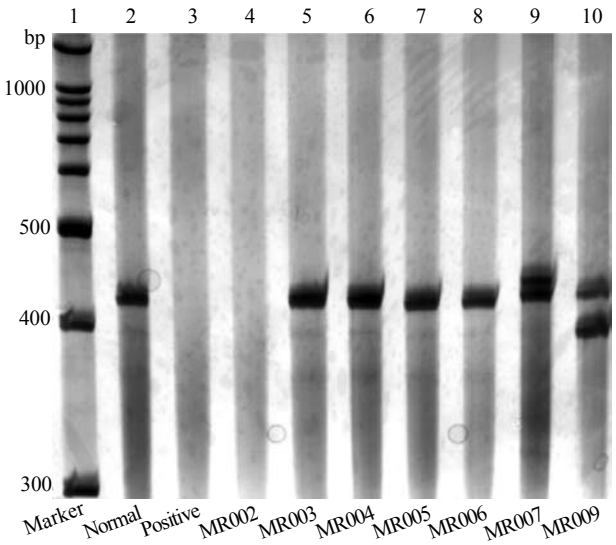

Fig. CGG repeats of FMR1 detected on the PAGE. Lane 1: 100 bp ladder; lane 2: normal control; lane 3: positive control; lanes 4-10: DNA samples from MR patients.

band (Fig.) (lanes 3 and 4) in male or there is only one band that can be detected in the DNA from females, we suspect large premutation or full mutation, for which oligo-(CGG)5 probe should be applied.[10,11] This is the case in the nine samples of this study. One FXS (2%) of the 50 cases supports our earlier finding that 2.8%-3.2% of Chinese mental retardation cases are associated with FXS.[5]

An overlapped phenotype was found between FXS and autism.[12,13] Patients with FXS may present autistic behavior. Among autism patients, 2%-6% of FXS mutations can be detected. To investigate whether the FXS mutation of CGG repeat expansion may present in Chinese autism patients, we analyzed 185 samples of autism DNA and found that 4.3% (8/185) DNA samples showed CGG repeat expansion. Although the underlying neurobiological mechanism of autistic behavior in FXS, which causes confusion in clinical practice between FXS and autism, is not known, the data support our hypothesis that FXS and autism may share a common pathogenic and neurobiological pathway. Further investigation on this unknown pathway may open a new avenue for treating both FXS and autism.

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