

DNA X

가 , *

=ABSTRACT=

Prenatal Diagnosis of Fragile X Syndrome using Amniotic Fluid DNA

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Background: The fragile X syndrome is the most common cause of inherited mental retardation, is almost always caused by abnormal CGG trinucleotide amplication within the FMR1(fragile X mental retardation) gene located in Xq27.3

Methods: DNA samples were obtained from the amniotic fluids of known carrier mother and 35 mothers without risk factors of the fragile X syndrome. Polymerase chain reaction(PCR) and Southern blot analysis were performed to evaluate the number of CGG repeats in the FMR1 gene.

Results: The DNA samples from the carrier mother gave a large fragment over 300 repeats by PCR. All 35 control samples showed fragments sized under 35 repeats.

Conclusion: Prenatal diagnosis of the fragile X syndrome could be done with mid-trimester amniotic fluid using PCR and Southern blot method.

Key words : Fragile X syndrome, Prenatal diagnosis

X , 가 (Xq 27.3)
 X 4. 1991
 , 4,000 가(FMR1 gene)
 6,000 가 1,2 3,5,6
 X (Xq27.3)
 FMR1 (FRAXA) 5' , ACOG
 CGG , 가
 FMR1 , 가 200 가 X 가
 , DNA
 3
 X
 X

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1. 가 32 가 가 X (biparietal diameter), (abdominal circumference) (femur length)가 19 , 25 15 , 30 ml 7 , 3.32 Kg 35 X , 가 가 가 2) DNA (pellet) X buffy coat (2,000 rpm, 10 ,) , (3,000rpm, 15 ,) (pellet) genomic DNA DNA 500 μ l DNA (lysis buffer; NaCl 0.25 M, Tris-HCl 50 mM/pH8.0, EDTA 5 mM, 0.5% SDS, Proteinase K 4 mg/ml ; Sigma) 가 37 3 phenol(0.1 M Tris-saturated phenol/pH7.0; GIFCO-BRL, Grand Island, NY. USA) 가 15,000 rpm 10 (microcentrifuge 5415 C, Effen-dorf, Engelsdorf, Germany) , phenol/chloroform/isoamylalcohol(25:24:1) 가 15,000 rpm 10 chloroform 가 3 M sodium acetate/pH5.2 0.1 volume 2.0 volume -20 1 4 10 15,000 rpm (pellet) 70% 3 PCR(polymerase chain reaction, PCR) Southern 가 35 X (Spectrophotomer-DU650; Beckman, Somerset, NJ. USA) DNA 0.1-1 ug DNA

3) PCR
 Master mixture solution
 , 2X GC rich modified buffer, 2.5 mM dNTPs
 (modified), Taq DNA polymerase, primer Bioneer
 (Fx5 : 5'-TGACGGAGGCGCCGCTGCCAGGGGGCGTG-3'),
 (Fx3 : 5'-CCAGCTCCTCCATCTTCTTCAGCCCTGC-3')
 master mixture solution .

PCR
 DNA 1 µg/µl
 . PCR 95 60 (dena-
 turation) , 65 60 (annealing)
 72 120 (elongation)
 35 .

30% acrylamide stock solution, 10X TBE buffer, TEMED
 (N,N,N',N'-Tetramethyl ethylenediamine) (Progma, USA),
 10% ammonium persulfate, 10%
 acrylamide gel , PCR 4µl
 (loading) 125 volt 3 .

gel ethidium bromide(Sigma; st Louis,
 MO USA) 10

(4) Southern

DNA
 genomic DNA 5µg/µl 10X EagI buffer
 EcoRI 가 37 3
 EagI 가 (mineral oil)
 50 . genomic
 DNA 1% agarose gel 30 volt
 (transfer) .

Blotting
 acrylamide gel transfer kit(SEMI-PHOR™, Hoeher Scien-
 tific Instruments) (Hybond-Nylon membrane,
 Amersham, USA) Whatman 3MM filter paper
 30 volt 4 80
 30 membrane

(probe)
 DNA (CGG₁₂) kit
 . DNA 가

10 ng/µl 100 5
 5 spin down 10 µl
 , Gene images™(Amersham,
 USA) Kit labelling reagent(containing 0.1% sodium azide)
 2 µl 10 µl crosslinker working solution
 (containing 4.7% formaldehyde) spin
 down 37 30 .
 (hybridization)
 55 wrap
 membrane 가 , 55 shaking water bath
 15 (prehybridization) .
 hybridization membrane 55
 shaking water bath . 1
 55 membrane 10
 2 . membrane 2
 30 .
 2 detection reagent
 (CDP-Star™ detection reagent, Amersham, USA) mem-
 brane 2-5 detection reagent
 saran wrap membrane 가
 (Hyperfilm, TM ECL) 1
 가
 (band) .

2. PCR (Fig. 1)
 X band가
 (1 lane). (lane 2) 300
 가
 DNA

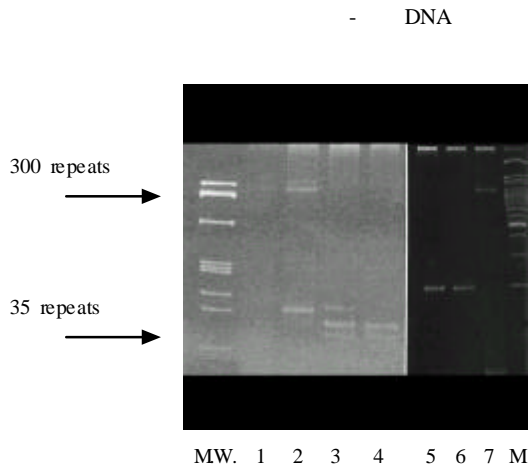


Fig. 1. Amplification of FMR1 gene by PCR
 MW.; size marker(phix174/HaeIII), lane 1 fragile patient,
 lane 2; mental retardation patient, lane 3-6; normal,
 lane 7; amniotic fluid of carrier, M; 100bp ladder.

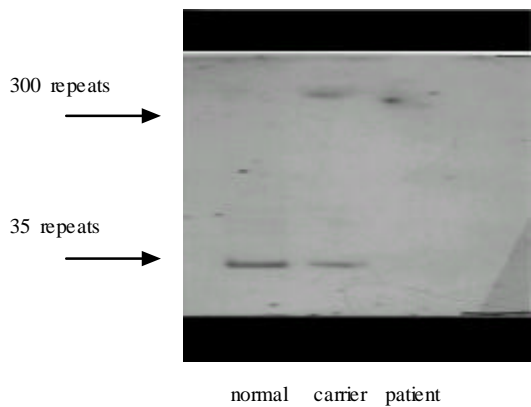


Fig. 2. Southern blot of FMR1 gene.

band가 (lane 3,4,5,6).
 DNA PCR , 300
 band가 (7 lane).
 CGG 가 15 -25
 band가
 가 1 ; 25-30 band가
 가 5 ; 15-25 , 30-35 band가
 가 5 ; 25-30 , 30-35 band
 가 가 24 .

3. Southern (Fig. 2)
 DNA (Fig. 2 normal lane),
 CGG
 가 300
 band band가 (Fig. 2)

X
 carrier lane). X
 가 300 band가 (Fig. 2 patient
 lane).

1943 Martin Bell 가
 , 1969 Lub Marker chromosome 가
 . 8 thymidine 가
 X
 4
 1991 X FMR1
 CGG . 3,5,6

X 1980 1:1,000^{9,10}
 - 1:2,600¹¹ 1:5,000,
 1:700

1:6,000² 가 1:259 1:4,000¹
 . 12 X 1-4%
 가

, 13 Xq27.3 가 20 14
 . X 15

2.8% 16
 X 가 35-55
 가 20-40 .

17
 (full mutation) ,

18 FMRP(X
) 60%가
 52%-82%

FMRP (I.Q < 85) 18
 heterozygotes X
 FMR1 allele 19
 가 20% ,
 16

- DNA X -

가 X 가 , 가 , X

X Xq27.3(FRAXA)

FRAXA FRAXE²¹ CGG

FRAXF X 가 300 X 35

X CGG

(PCR) Southern Southern 가 가 가

Hind , Bg , Pst 50% , 50%

가

PCR 150 가

가²⁰ PCR 가

band가 , 가

Southern band , 가

digoxigenin expand PCR 가 X

²⁹ , , , , , ,

PCR Southern 가 가

가¹⁹ 가

FMR1 70-80 k Dalton FMR 가 ,

. FMR

²⁰ ,

¹⁸ 가

X 가 가

가 30% , 40% 가 가

가²⁰ 20%, 5% 가

(lyonisation)

80% , 80% ,

40% ,

²⁰ ,

X 가 가 X 가

1 , , 가 , X

가 가 FMR

50% 가 FMR

가

X

FMR
가
PCR Southern
X
PCR
가 , FMR 1
가

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