

Fragile X Mental Retardation in an Indonesian Family

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Abstrak

Sindroma fragile X adalah penyakit retardasi mental menurun yang paling umum dan dikaitkan dengan adanya "fragile site" pada ujung akhir lengan panjang kromosom X. Dalam penelitian penyaringan untuk mencari kelainan kromosom pada siswa laki-laki retardasi mental dari sekolah luar-biasa, kami pertama kali mengidentifikasi keluarga dengan sindroma fragile X di Indonesia. Pada pemeriksaan fisik, riwayat keluarga dan pemeriksaan sitogenetika dan DNA membuktikan dua kakak beradik laki-laki dengan fenotip abnormal, positif fragile X masing-masing 3% (Dm) dan 4% (Pr). Kakak perempuan dan ibunya menunjukkan fenotip dan intelektual normal, tetapi secara sitogenetik positif untuk fragile X (6% pada kakak perempuan dan 3% pada ibunya). Analisis molekuler meyakinkan bahwa kedua anak laki-laki tersebut adalah penderita sindroma fragile X, sedangkan kakak perempuan dan ibunya adalah pembawa sifat.

Abstract

Fragile X syndrome is the most common form of familial mental retardation and is associated with a fragile site at the end of long arm chromosome X. Whilst screening for chromosomal abnormalities in mentally retarded males attending special school, we have identified the first family with a fragile X syndrome, to be reported in Indonesia. Physical examination, family history, as well as cytogenetic and DNA studies identified two phenotypically abnormal brothers who were fragile X positive 3% (Dm) and 4% (Pr). Their sister (Cl) and mother were phenotypically and intellectually normal but were cytogenetically positive for fragile X (6% in the sister and 3% in the mother). Molecular analysis confirmed that both brothers were affected and that their sister and mother were carriers.

Keywords : Fragile X syndrome, mental retardation, Indonesia, Javanese, chromosome studies, restriction fragment length polymorphism, pfxa3.

INTRODUCTION

Mental retardation (MR) is a common disease which is clinically and etiologically heterogenous. MR can be divided up according to intelligence quotient (IQ) test scores into severe or moderate (IQ 50 or less) and mild (IQ 50-70),¹⁻³ with a prevalence of about 3 per 1000 for severe mental retardation and 30 per 1000 for mild mental retardation.² Causes of mental retardation include obstetric problems, perinatal trauma, neonatal infection and genetic abnormalities such as Down's syndrome (trisomy 21) and X-linked mental retardation (XLMR). Almost all surveys of mentally retarded persons show that males tend affected more often than females. Of all mentally retarded people, 20% are due

to XLMR.⁴⁻⁶ About 40% of XLMR and 4% of all mental retardation have been attributed to fragile X syndrome.^{1,6} Fragile X syndrome is the most frequent known inherited cause of mental retardation, with an estimated prevalence in predominantly Caucasian populations ranging from 0.4/1000 to 0.8/1000 in males and from 0.2/1000 to 0.6/1000 in females.^{4,7}

Affected individuals have minor physical abnormalities and may fail to thrive in infancy or childhood or have learning difficulties in school. These children have a reasonably long life span, but require constant care from either the family or an institution. They usually attend special schools for the mentally handicapped. Fragile X syndrome is characterized by a cytogenetically detectable fragile site which appears as

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a break near the end of the long arm of the X chromosome. The basic defect of fragile X is expansion of the number of the trinucleotide CGG repeats at the 5' end of the FRAXA gene.⁸

Chromosomal disorders in mentally retarded children have not been studied in Indonesia because of the lack of diagnostic facilities. While undertaking a survey of chromosomal abnormalities in mentally retarded children in Semarang, Indonesia, we identified a family with two mentally retarded males demonstrating the presence of fragile X chromosomes. This is the first reported family with fragile X in Indonesia.

MATERIALS AND METHODS

Patients

The family was identified in the course of a screening program of MR males who appeared to be phenotypically normal. There were two brothers (Dm age 13 and Pr age 8) in a special school, reported to be intellectually handicapped. They have one sister (Cl age 14) who is intellectually normal and attends a normal school. Both parents are alive and well (see pedigree). Findings from a physical examination including testicular measurement (comparative palpation) using testicular models of known volume (orchidometer) of these two brothers are shown in Table 1.

Table 1. Clinical description of the two brothers.

	Case 1 (Dm)	Case 2 (Pr)
Date of birth	27/3/1981	18/8/1986
Age at time of examination	13 years	8 years
Head Circumference	58 cm(92%)	53cm(>95%)
Weight	49kg	19kg
Hcight	158cm(95%)	112cm(95%)
Long face	Yes	Yes
High forehead	Yes	Yes
Prognathism	Yes	Yes
Long & prominent ears	Yes	Yes
Macroorchidism (testicular volume)	Yes(>25ml)	Yes(4ml)
Strabismus	Yes	Yes
Nystagmus	Yes	No
Cogwheel rigidity	Yes	Yes
Abnormal gait & parkinsonian tremor	Yes	No
Stereotypic movement	Yes	No
Hyperactive	No	Yes
Autism	Yes	No
Shyness	Yes	Yes
Gaze avoidance	Yes	Yes
Language difficulties	Yes	Yes

Blood collection

Ten ml of heparinized peripheral blood were drawn from each individual for cytogenetic and DNA analysis. Blood was also screened for hypothyroidism by blotting it on special filter paper.

Cytogenetic studies

Chromosome preparations were made by culturing 10 drops of peripheral blood at 37°C for 72 hours in duplicate tubes each containing 5 ml Iscove's Low Folate medium supplemented with 5% Fetal Bovine Serum (FBS) and 0.025 ml Phytohaemagglutinin-P (Gibco). To each tube, 0.1 ml thymidine (final concentration 0.3 µg/ml) and 3 drops colchicine (final concentration 1 µg/ml) were added respectively at 24 hours and 25 minutes before harvesting. Chromosome spreads were made according to routine procedures. Chromosome analysis for fragile X was performed by scanning 50 unbanded Giemsa-stained metaphases. When cells with chromosome breakage in group C were found, a further 50 cells and 100 cells were counted in males and females respectively. The coordinates of positive cells were noted and the metaphases photographed. Subsequently, the slides were destained in 3:1 methanol:acetic acid, and G-banded for confirmation of the fragile X. Six further new banded cells were analyzed and another 20 cells were counted to assess structural and numerical chromosome abnormalities (Figure 1 shows one chromosome with fragile X in Cl).

Molecular studies

DNA was extracted from peripheral blood by using the saturated salt method.¹⁰ Eight micrograms of DNA were digested with one unit of the restriction endonuclease *Pst*I (Pharmacia) in the buffer recommended by the manufacturer together with spermidine in a total volume of 20 µl. Agarose gel electrophoresis was performed (0.75% agarose, x1 Tris-borate EDTA buffer, 35 volts for 16 hours). An SPP1 ladder (Bresatec) and normal control DNA were included in each gel. After capillary blotting to Hybond N⁺ (Amersham), prehybridisation was performed for one hour in rapid-hyb buffer (Amersham RPN 1636) at 65°C. The FRAXA probe pfxa3 and an X-chromosome single locus control probe pS8 (both kindly supplied by Professor G. Sutherland, Adelaide) were prepared by ³²P-incorporation by random hexamer priming, and hybridisation performed in the same buffer for 16 hours at 65°C. Washing was performed in 2x SSC and 0.1% sodium dodecylsulphate (SDS) three times at



Figure 1. Fragile X chromosome (left) and normal X chromosome (right) in female (cl)

room temperature for 20 minutes and then twice for 30 minutes at 65°C in 0.1x SSC and 0.1% SDS. Autoradiography was performed for 1-3 days at -70°C using intensification screens.

Screening for hypothyroidism

Blood was blotted on Whatman filter paper (S&S 903 LOT#W-621), dried and screened for hypothyroidism by Dr. Bridget Wilcken from the Oliver Latham Laboratory, Sydney.

RESULTS

Clinical finding

A family of five (2 parents and 3 siblings) was studied. Two affected brothers were examined. Both brothers had no obstetric and neonatal problems as well as stigmata of hypothyroidism. Their parents and sister were normal on physical examination. The pedigree is shown in Figure 2 and clinical features of the two brothers are presented in Table 1 and Figure 3.

Cytogenetic Analysis

Cytogenetic study of two affected sons identified a break in the long arm of the X chromosome at q 27.3 (FRAXA locus) in 100 metaphases: 3% in Dm and 4% in Pr. The mother and daughter (Cl) had 3% and 6%

fragile X cells in 150 metaphases respectively. No fragile sites were identified in 50 metaphases in the father.

Molecular Analysis

The Restriction fragment length polymorphism (RFLP) using the pfxa3 probe detected a constant band between 1.0 and 1.1 kb in normal controls. Simultaneous hybridisation with pS8 yielded a 0.8 kb band which provided an intensity control for normal X-chromosome copy number in all individuals.

In proband son I (Dm) the normal pfxa band was replaced by 1.60 kb and 2.6 kb bands, while his brother son II (Pr) had a 2.2 kb but no normal band. Their sister (Cl) was a carrier, with an abnormal 2.2 kb and a normal 1.1 kb band which was also present in their father. Their mother was also a carrier, with abnormal 1.3 kb and normal 1.1 kb bands (see Figure 2).

Screening for hypothyroidism

Screening for hypothyroidism was negative for all five members of family.

DISCUSSION

We report the first recorded cases of Fragile X in an Indonesian family of Javanese ethnic background. We are not aware of any previous recorded cases in In-

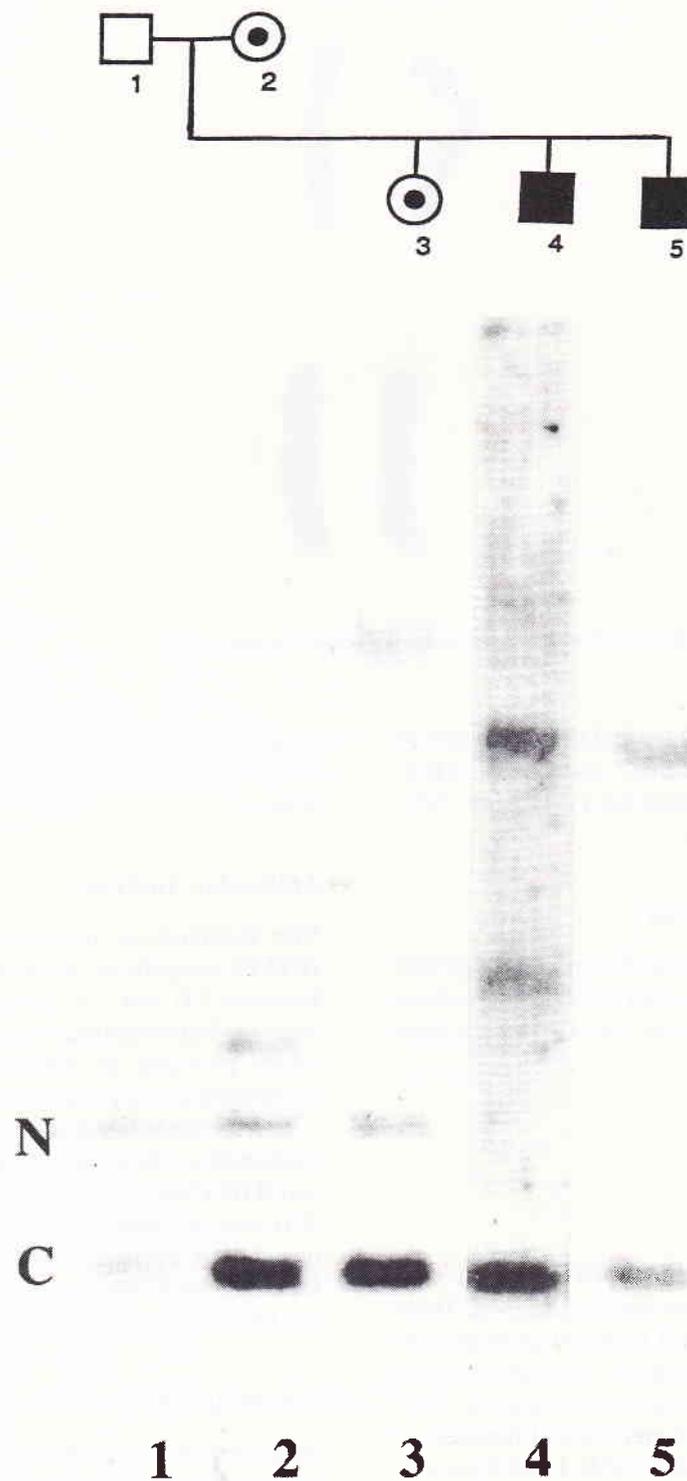


Figure 2. Shows five lines of a Southern blot. The band obtained by hybridising PstI digested genomic DNA with the X chromosome probe pS8 is used as a control (C) for densitometry, while the normal band size (N) obtained using the FRAXA probe is indicated. The father (lane 1) has only a normal band, while the mother (lane 2) is a carrier, since there is an additional higher band. Each FRAXA band is of reduced density relative to the control, C. The daughter (lane 3) is also a carrier, while the sons Dm (lane 4) and Pr (lane 5) lack the normal band, which has been replaced by two and one higher band respectively, they are therefore affected individuals.

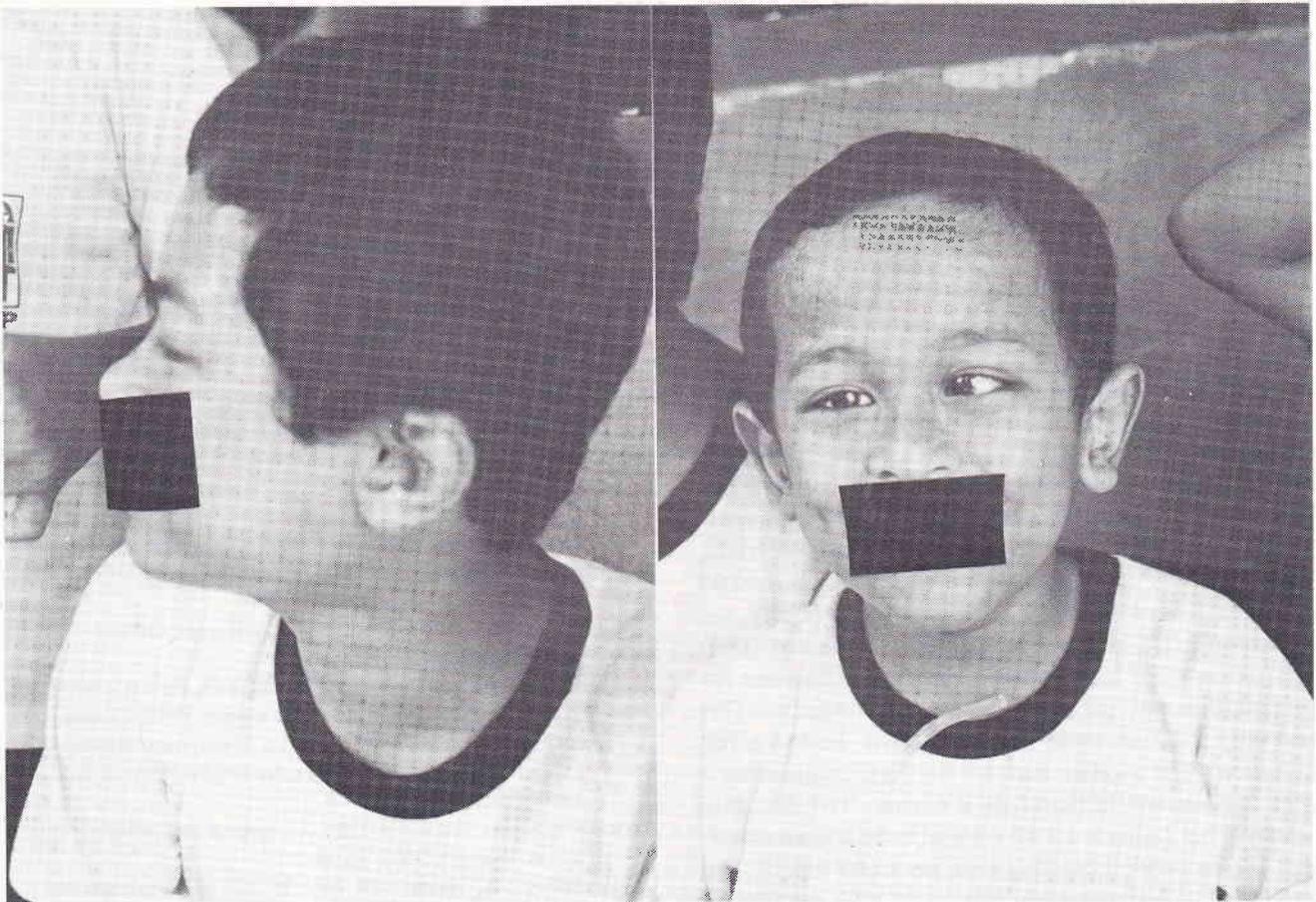


Figure 3. Two affected brothers, Dm (left) and Pr (right)

onesia except for a case of fragile X mental retardation in one Dutch-Indonesian in Hawaii, whose ethnic background was not reported.¹¹

The affected males (Dm and Pr) showed macroorchidism. In the case of the 8 year old the testicular volume should be less than 4 ml. In the 13 years old who is showing early sign of puberty such as some pubic hair the testicular size should be 4 ml but is already over 25 ml. Both have long ears and prominent jaws, shyness, no speech difficulties but both have language deficits such as abnormally frequent use of short sentences. These features were present in many patients of fragile X syndrome.^{4,7,12} They also had strabismus and nystagmus which are occasional fea-

tures in these individuals.^{4,13} The two affected boys have different characters: one is hyperactive and the other quiet and autistic. Both traits are seen in fragile X MR syndrome

The frequency of fragile X cells is 3% - 4% in the affected males (two sons) appear to be lower than in other studies which reported an incidence of 2-50%.^{1,7} Interestingly, the frequency of fragile cells was higher in the carrier daughter than in her affected brothers.

The fragile X locus is a folate sensitive fragile site. Detection is dependent on either a culture medium deficient in folate or containing an inhibitor of folate metabolism (eg.FUdR, methotrexate, excess

thymidine) and reduced content of FBS.^{14,15,16} It is possible that although low folate medium was used there was enough folate and FBS in the medium to reduce the incidence of fragile X expression. Recently two other folate sensitive fragile sites (FRAXE and FRAXF) were found in mentally retarded cases at Xq27-28.^{17,18,19,20} These new rare fragile sites can only be distinguished from the fragile X by DNA analysis.

DNA analysis using the probe pfxa3 and the restriction enzyme *Pst* I is diagnostic of the fragile X genotype. The band size reflects the number of CGG repeats with the following finding: in non-carriers (male or female) there is a constant band of 1.0-1.1 kb; in phenotypically normal male carriers, there is a band between 1.1 and 1.6 kb in size representing 70-200 CGG copies.^{16,21,22} In affected males there are larger and/or multiple bands with the size of the band greater than about 1.6 kb (>200copies) together with hypermethylation of CpG dinucleotides. In female carriers, 70-200 copies are designated premutation and >200 copies a full mutation.^{16,19,23} In our case family, we identified a 1.1 kb band in the father. One son (Dm) had 1.6 and 2.6 kb bands suggesting mosaicism for premutation and full mutation, while the other son (Pr) had only a 2.2 kb band indicating that he had a full mutation. The mother had 1.3 kb band suggesting a premutation and is therefore a carrier. The daughter who had her father's 1.1 kb normal band and an abnormal 2.2 kb band, indicating that her CGG repeats had expanded to full mutation, consistent with the finding of others in the literature that there is a progression of expansion from one generation to the next.^{2,19,22}

The implications of these findings are that other members of the family, in particular aunts and uncles of the proposita should be studied to determine the origin of the fragile X. Genetic counseling can then be promoted for members of the family who have been investigated.

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