

*Full Length Research Paper*

# **Fragile X allelemorphism among the mentally retarded and in affected families**

**A. Latunji Charles**

Cell Biology and Molecular Genetics Unit, Zoology Department, University of Ibadan, Ibadan, Nigeria. E-mail: [ca.latunji@ui.edu.ng](mailto:ca.latunji@ui.edu.ng). Tel: 234-8055200995.

Accepted 11 September, 2009

**The fragile X mental retardation allele was investigated in Ibadan, south west Nigeria. Blood specimens from a population of 659 mentally retarded individuals (MRs) were screened for the fragile X mutation using cytogenetic and molecular methods. It was observed that 235 (35.7%) individuals had chromosomal aetiologies to their mental impairment. The Down syndrome was highest occurring in 146 (21.2%) individuals, followed by the fragile X karyotype with 45 (6.83%) individuals expressing the 46, Xq27.3 fragility. Of the fragile X individuals, there were 13 (2.0%) fragile X females, 2 of which were 45, XO/46, XX and 45, XO/46, XX/47, XXX mosaics, respectively. Molecular methods confirmed the cytogenetic findings, where affected individuals expressed the trinucleotide repeat amplification in the order of > 2 00 CGG repeats in the fragile X allele region. Triplet repeat bands ranged between 200 and 2000 CGGs. Eight pedigrees comprising 70 normal relatives of 8 fragile X mentally retarded propositi were permissible to investigations for the determination of interfamilial transmission of the fragile X alleles. Blood samples were equally obtained from them and analysed using cytogenetic and molecular methods likewise. Two normal sisters of a male propositus exhibited the 45, XO/47, XXX and 46, Xr(X)/45, XO mosaicism, respectively. Molecular analysis revealed 26 (33.3%) female permutation carriers and 11 (14.1%) normal transmitting males. Eighteen (23.1%) males had normal alleles, thus are non-transmitting males, and 15 (19.2%) females were normal. The proportions observed in this survey have implications for the general population and should prove significantly useful for clinicians and genetic counsellors.**

**Key words:** Fragile X syndrome, mental retardation, karyotype, cytogenetic, molecular analysis.

## **INTRODUCTION**

Fragile X syndrome (FXS) is still the highest cause of inherited mental retardation (MR) and accounts for perhaps 2 to 4% of mental retardation overall. It is second only to Down syndrome as a specific cause of MR (Brown, 1995). The designation fragile X is based on the presence of a gap or break at the end of the one arm of the X chromosome when affected cells are cultured in special media (Sutherland et al., 1985). Approximately, 95% of adult fragile X males have an intelligent quotient (IQ) below 70 with overall mean of 35 (Shiue et al., 2004; Sutherland et al., 1985). Fragile X syndrome also appears to show significant association with infantile autism (Belmonte et al., 2004; Blanc et al., 1993). Autistic individuals are usually males and a 4:1 male to female ratio is commonly observed. About one in five males who

inherit the fragile X chromosome are normal carrier males (Sherman et al., 1985). These males are non-penetrant for the mutation and do not express the fragile site. They transmit the mutation to their daughters who are also non-expressing for the mutation but who can then have affected sons. Majority of female permutation carriers are mentally normal. Approximately one in three females have some evidence of mental impairment and about one in ten is considered mentally retarded. A characteristic profile of cognitive defects in affected carriers may be present (Brown, 1995). The syndrome and its particular pattern of heredity are caused by a dynamic mutation, involving an unstable expansion of a trinucleotide (CGG) repeat at the 5' UTR (5' untranslated region) of the fragile X-mental retardation (FMR1) gene, located at Xq27.3.

Expansion of this repeat region greater than 200 repeats leads to methylation-coupled silencing of the gene and absence of the fragile X mental retardation protein (FMRP), causing the classical FXS (Van Esch et al., 2005). Individuals with expanded repeat lengths varying from 50 to 200 repeats do not exhibit the classical FXS phenotype, but are considered as fragile X premutation carriers (Van Esch et al., 2005).

The FMR1 gene is expressed early in development. With maturation, it is expressed strongly in the brain, gonad, oesophagus, thymus, spleen and lymphocytes, weakly in the liver, kidney, colon, thyroid and lung, but not at all in muscle, heart and aorta (Hinds et al., 1993).

Although, studies on the FXS abound in literature from many populations, the author is oblivious of any prior publication on the prevalence of the FXS from a Negroid African cohort population. As before, compared to individuals diagnosed with most other genetic or chromosomal syndromes, affected fragile X males are usually fairly normal in physical appearance and therefore escape detection earlier in life. This paper reports, the prevalence of the FXS among mentally retarded individuals (MRs) and examines the intra-familial dynamics of the FMR1 allele transmission.

## MATERIALS AND METHODS

### Subjects

Mentally retarded individuals (MRs) were sought from schools/homes for the mentally handicapped in the city of Ibadan and environs. The criteria for recruitment into this study were mental retardation and evidence of registration in these special schools/homes for the mentally retarded. Peripheral blood specimen was obtained via vein-puncture aseptically from the MRs in a continuous exercise and as they become available between 1996 and 2001. All subjects ranged from mildly to severely retarded. Equal volume of blood was withdrawn from mentally normal individuals of similar age distribution and used as controls.

Familial investigation was routinely sought from all FXS individuals and where permissible blood specimen was withdrawn from volunteer family members. The criterion for inclusion in this part of the study was familial relationship.

### Constitutive karyology

Phytohaemagglutinin (PHA) stimulated whole blood microculture technique {modification of the technique of Dewald et al. (1992) was used to cultivate the lymphocytes of each donor}. 0.2 ml whole blood inoculum was injected into 5 ml of medium 199 for fragile X (sigma) supplemented with pooled human AB+ve serum, gentamicine, L-glutamine and PHA. Cultures were incubated at 37°C for 72 h and harvested. Vincristine sulphate (a spindle inhibitor) was introduced at 70 h (0.002 µg/ml of culture) and at 72 h cultures were spun down at 5000 rpm for 3 min. The pellets were hypotonised in 0.075 M KCl solution for 8 min spun down again and re-suspended in fresh cold fixative (3:1 methanol: glacial acetic acid). Slides were prepared and analysed microscopically. A minimum of 50 non-banded Giemsa stained cells and 10 Giemsa-trypsin banded cells were scored for the fragile X. Numerical aberrations encountered were recorded and banding was used primarily to distinguish the X-chromosome from other group C chromosomes.

### DNA isolation

Blood spotted on blotting papers and dried were lysed in sodium dodecyl sulphate (SDS) – perchlorate, digested overnight with proteinase K and isolated according to the procedure of (Strauss, 1988). The DNA from all samples was extracted with chloroform and precipitated with cold ethanol.

### PCR amplification

Amplifications were carried out in a thermalcycler in 40 µl reactions, containing buffer (10 mmol/l tromethamine hydrochloride, pH 8.3, 50 mmol/l potassium chloride), 1.25 U Taq polymerase, 10% dimethyl sulfoxide (DMSO), 25 pmol of each primer and approximately 200 ng of template DNA. The dNTPs were 200 µmol/L each of dATP, ACTP, DTTP and 7-deaza-2-deoxyguano-sine triphosphate (7-deaza-dGTP). Magnesium chloride concentration used was 1.25 mmol/L.

The PCR primers used were (5'-GAC GGA GGC GCC GCT GCC AGG- 3') (invitrogen) for the forward amplification and (5' –TCC TCC ATC TTC TCT TCA GCC CT -3') (invitrogen) for the reverse amplification. The primers annealed at 61°C. Denaturations were performed at 94°C to optimise product yield of disparate size alleles (Walsh, 1992).

### Electrophoresis and gel transfer

5 µl of PCR product were combined with 5 µl of loading buffer (80% formamide, 0.1% bromophenolblue, 0.1 Xylene cyanol) and separated by electrophoresis through 6% polyacrilamide, 8.3 mol/l urea denaturing gel with TBE running buffer at 500 V for 60 min, then transferred to positively charged nylon filters by electroblothing (Trnovski, 1992) for 30 min using semi dry electroblotter at 3 mA/cm<sup>2</sup> constant current.

### Chemiluminescence probe analysis

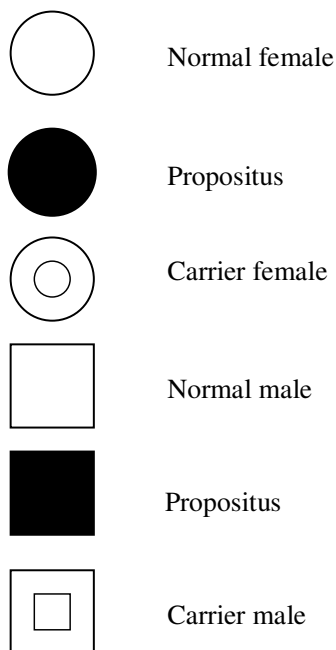
For the detection of PCR products, a hybridising oligonucleotide probe producing chemiluminescence was used (Pollard-Knight, 1990). Probe, chemiluminiscent spray and hybridising agents were supplied as a kit (invitrogen). A (CGG)-5' probe labelled with alkaline phosphatase was hybridised to the nylon membranes for 20 min at 55°C, sprayed with chemiluminiscent spray, sealed in plastic folders and exposed to roentgenogram film at 37°C for 60 min and overnight.

## RESULTS

The MRs studied was aged between 12 and 39 years old and they ranged from mildly to severely retarded. They all attended special schools/homes for the mentally handicapped. A total of 659 samples collected over a 5 year period (1996 – 2001) were screened cytogenetically for the fragile X chromosome. The summary of the cytogenetic result presented in Table 1 showed that 32.6% had chromosomal aneuploidies as the aetiologies of their conditions. Down syndrome was highest in occurrence among the MRs of 22.2%, followed by the FXS with a frequency of 6.8% individuals expressing the Xq27.3 X-chromosome fragility. Two (15.4%) FXS females exhibited cytogenetic mosaicisms of 45XO/46XX and 45XO / 46XX / 47XXX karyotypes, respectively. In pedigree studies, 8

**Table 1.** Summary of fragile X allele prevalence and cytogenetic status of the mentally retarded individuals.

Status	No. of males (%)	No. of females (%)	Total
<b>CGG repeat</b>			
Normal	349 (53.0)	222 (33.7)	571
Premutation carriers	7 (1.06)	16 (2.43)	23
Affected MR	52 (7.90)	13 (1.97)	65
Total	408	251	659
<b>Karyotypic</b>			
46, XY	206 (31.3)	-	206
46, XX	-	238 (36.1)	238
46, XY fragile Xq27.3	32 (4.90)	-	32
46, XX fragile Xq27.3	-	11 (1.67)	11
47, XX+G21	-	59 (8.95)	59
47, XY+G21	87 (13.2)	-	87
47, XXY	7 (1.06)	-	7
45, XO	-	17 (2.58)	17
45, XO/46,XX fragile Xq27.3	-	1 (0.15)	1
45, XO/46, XX/47,XXX Fragile Xq27.3	-	1 (0.15)	1
Total	332	327	659



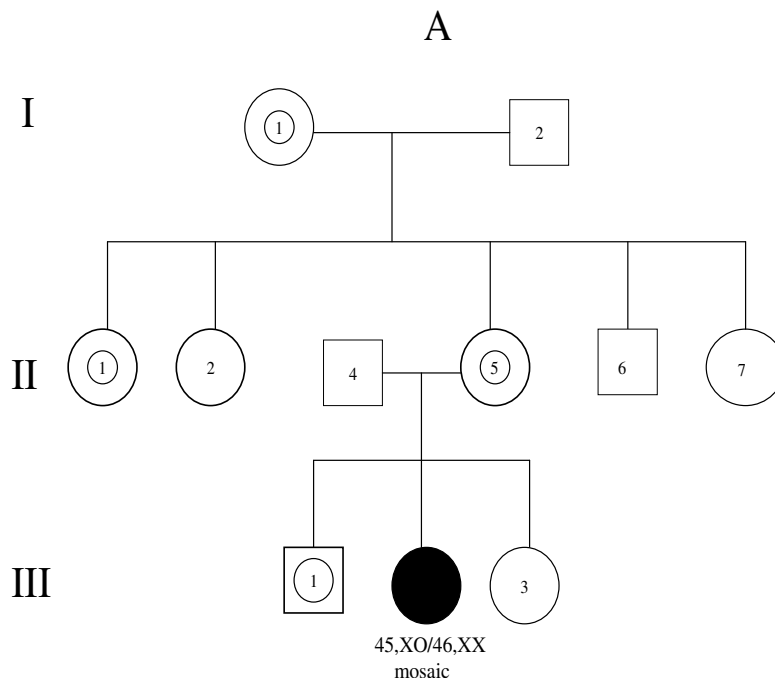
Key to Figures 1 to 8.

probands and 70 members of their respective families were permissible to investigation (Figures 1 - 8). These 70 volunteers were mentally normal relatives of 4 mentally retarded FXS males and 4 mentally retarded FXS females. The cytogenetic data of the 8 pedigrees as well as their fragile

X allele status is presented in Table 2. Two normal sisters of a male proband in family H (Figure 8) were observed to exhibit cytogenetic mosaicism one of whom was a numerico-structural mosaic of the 46Xr(X)/45/XO karyotype. Although other members of the pedigrees appeared to be cytogenetically normal, the molecular methods revealed some interesting features about the transmission pattern of the FMR1 allele. The premutation (PM) carrier status of individuals in the respective D pedigrees is shown in Table 3. The phenomenon of 'anticipation' and instability of the CGG repeat expansion with succeeding generation was observed in families and E (Figures 4 and 5, respectively). On the pedigree charts (Figures 1-8), daughters of unaffected transmitting males inherited the PM carrier status of the FXS condition, while males are likely to be affected only when there is an amplification of the repeat sequence trans-generational from their mothers. Daughters of carrier mothers also inherited the PM carrier status but not the full mutation with an exception in family H where there is a normal non-transmitting father and a normal PM carrier mother.

**DISCUSSION**

The different karyologies observed among this group of MRs (Table 1b) showed the FXS to be second only in frequency to trisomy G-21 (Down syndrome) as the most common cause of mental retardation with chromosomal aetiology. Screening populations for the fragile X chromosome has yielded heterogeneous results, depending on the selected criteria of subjects. Some studies in male



**Figure 1.** Family A with a female mosaic propositus who has normal father, a carrier mother and a normal grandfather

**Table 2.** Summary of fragile X allele and cytogenetic status in the pedigrees.

Status	No of males (%)	No. of females (%)	Total
<b>CGG repeat</b>			
Normal	23 (29.5)	16 (20.5)	39
Premutation carriers	5 (6.41)	26 (33.3)	31
Affected MR	4 (5.10)	4 (5.10)	8
<b>Total</b>	<b>32</b>	<b>46</b>	<b>78</b>
<b>Karyotypic</b>			
46, XY	28 (35.9)	-	28
46, XX	-	40 (51.3)	40
45, XO/46,XX	-	1 (1.30)	1
45, XO/46, XX/47, XXX	-	1 (1.30)	1
46, XX/47, XXX	-	1 (1.30)	1
46, Xr(X)/45, XO	-	1 (1.30)	1
46, XX fragile Xq27.3	-	2 (2.56)	2
46, XX fragile Xq27.3	4 (5.1)	-	4
<b>Total</b>	<b>32</b>	<b>46</b>	<b>78</b>

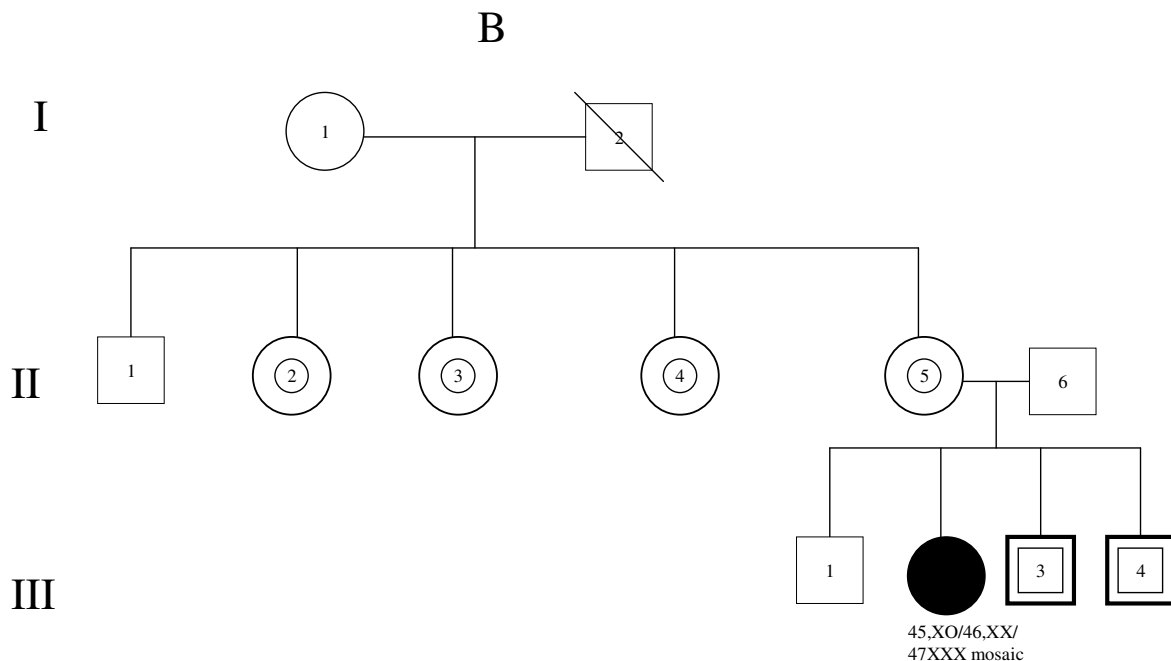
male populations selected individuals with macroorchidism for cytogenetic analysis (Brown et al., 1981; Howard-Peebles and Finley, 1983; Venter et al., 1984). Others selected male populations with familial mental retardation (Fryns et al., 1984; Venter et al., 1981).

These investigations showed the highest frequencies of males with FXS (Primrose et al., 1986). In most such studies the selection criterion was mental retardation not associated with chromosomal aberrations (Arinami et al.,

1987; Blomquist et al., 1983; Sutherland et al., 1985; Thake et al., 1987). Very few studies however included all individuals in a study group regardless of the cause of mental retardation (Jacobs et al., 1986; Kahkonen et al., 1986). In the case of males, macroorchidism is not always present. The choice of mentally defective population for this study removes this bias and much more random and reliable data could be obtained. The prevalence of FXS in males in institutions for the mentally

**Table 3.** Composition of the fragile X allele status in the pedigrees.

Pedigree	No. of normal males	No. of carrier females	No. of affected males	No. of normal females	No. of carrier females	No. of affected females	Total individuals
Figure 1	4	1	-	3	2	1	11
Figure 2	3	2	-	1	4	1	11
Figure 3	-	1	1	2	3	-	7
Figure 4	2	1	1	-	5	-	9
Figure 5	2	5	1	2	3	-	13
Figure 6	1	-	1	4	1	-	7
Figure 7	4	-	-	3	2	1	10
Figure 8	1	1	-	-	7	1	10
Total	17	11	4	15	27	4	78



**Figure 2.** Family B with a female mosaic propositus who had a normal father, a carrier mother and two normal transmitting brothers.

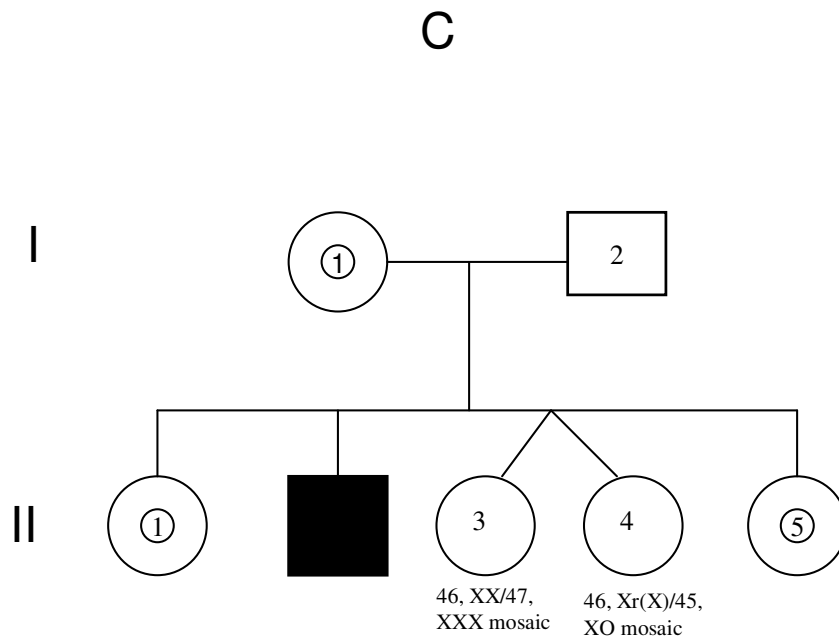
retarded was 1.6 - 6.0%, rising to 7 - 9% if known chromosomal or dysmorphic cases are excluded (Pembrey and Baraitser, 1986) with improved methodologies. In this particular study, the preponderance of males with the FXS among the MRs is 4.9% (Table 1b). The most significant impact of improved diagnostic tool was in the elimination of equivocal fragile X detection results. It is conjectured that since the criterion for screening is solely mental retardation and devoid of any known dysmorphic, chromosomal or familial bias, the proportions of unaffected carrier males and females observed in the pedigrees might have implications for the general population (Table 2).

The observed mosaic karyotypes and gel bands in the

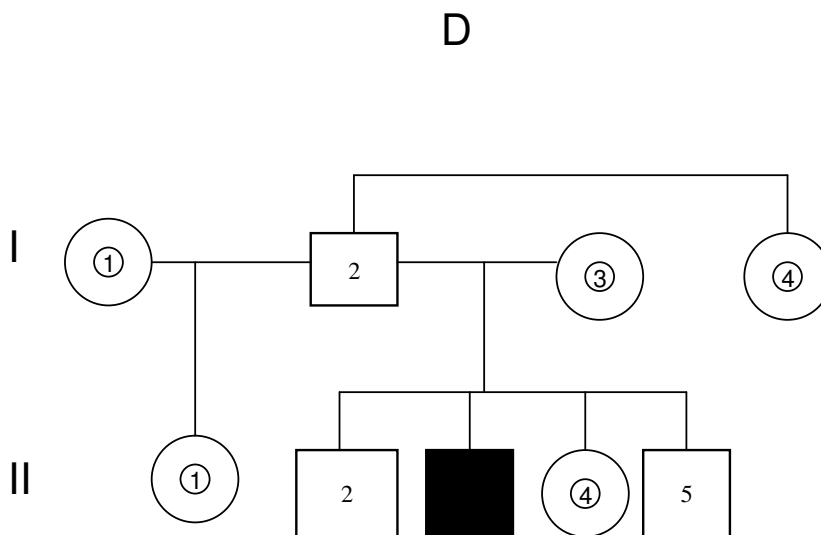
pedigree studies is indicative that this phenomenon is not uncommon with the inheritance of the FMR1 alleles.

Since the mutations of the CGG repeats are dynamic and may vary from generation to generation as well as within a single person during early embryogenesis (Devys et al., 1992; Wohrle et al., 1993), these premutation (PM) alleles may become unstable, only through maternal transmission, with further expansion in the next generations. For a long-time, male and female PM carriers were considered as asymptomatic. This view was, however, gradually challenged with the description and reports of different PM-associated clinical phenotypes over the last decade (Van Esch, 2006).

According to the standard model, the FMR1 gene be-



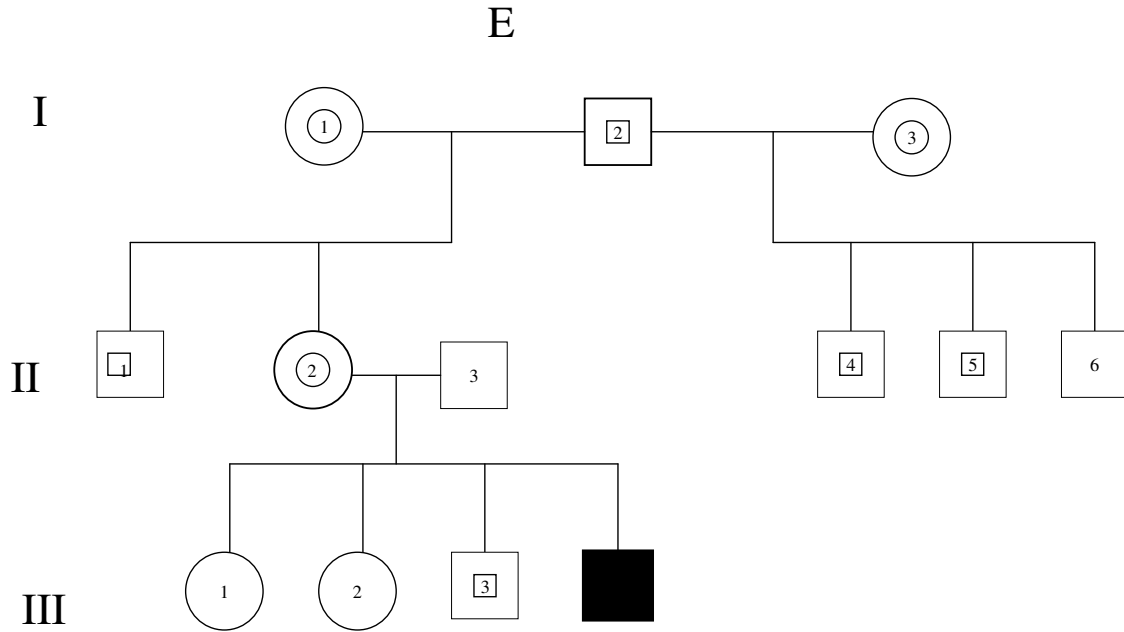
**Figure 3.** Pedigree of family C with a male proband who had two mentally normal cytogenetically mosaic sisters, a normal transmitting father and a carrier mother



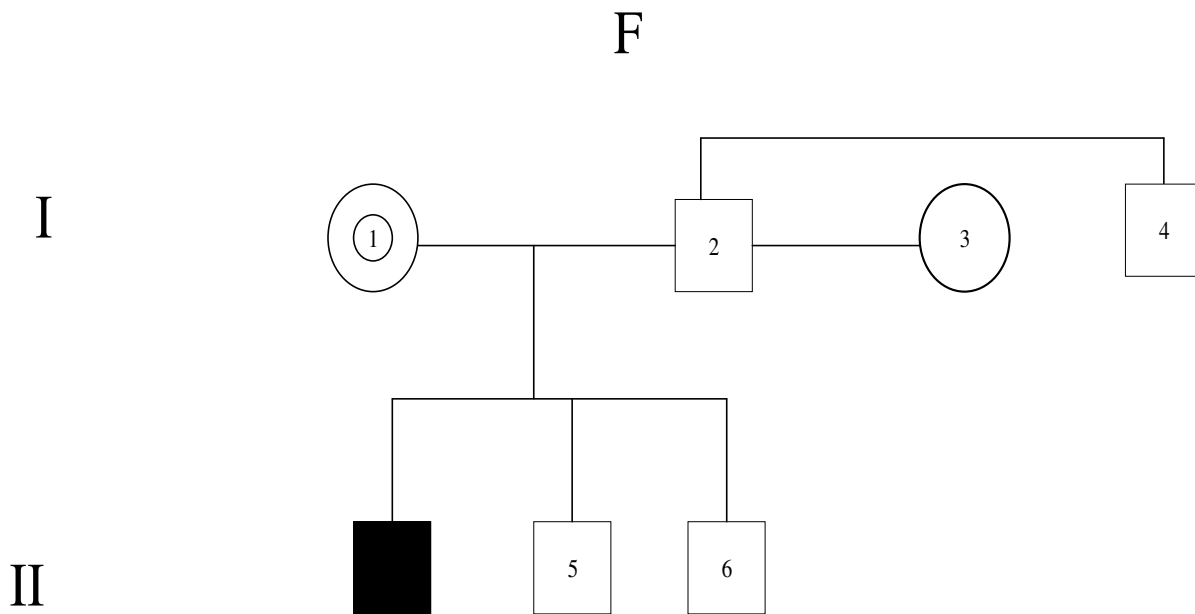
**Figure 4.** Pedigree of family D with a male proband who had a normal transmitting father and a carrier mother.

comes silenced as a consequence of the expansion and the methylation of the CGG trinucleotide repeat element located in the 5'-UTR of the gene (Pieretti et al., 1991). The resulting absence (or deficit) of the fragile X mental retardation protein (FMRP) causes FXS. Based on this model, carriers of the PM alleles were assumed clinically

normal (asymptomatic), since methylation-coupled silencing does not occur in the PM range. However, contrary to expectation, a subgroup of male and female carriers does show some clinical involvement which includes physical features, learning disabilities, anxiety, developmental delay and emotional problems. In addition, appro-



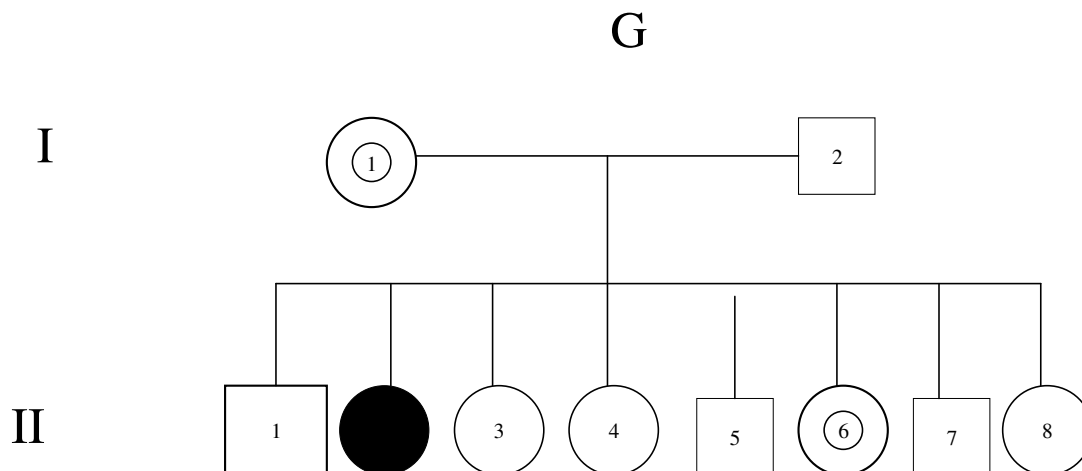
**Figure 5.** Family E with a male propositus who had a normal non-transmitting father, a carrier mother, and a normal transmitting grandfather.



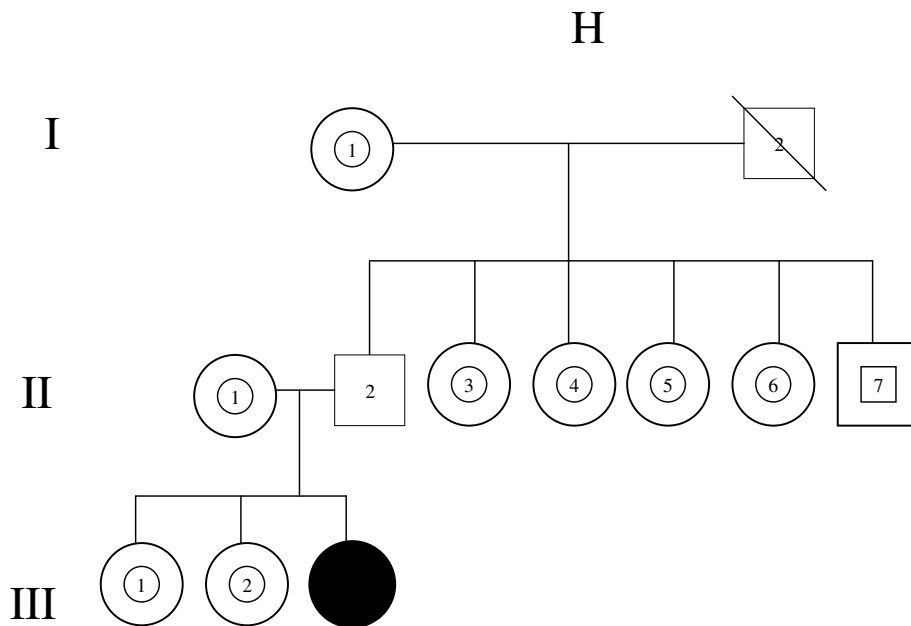
**Figure 6.** Family F with a male propositus who had a carrier mother and a normal non-transmitting father.

ximately 20% of female carriers of PM alleles have premature ovarian failure, which is not observed in females with hypermethylated full mutation (Allingham-Hawkins et al., 1999). This may explain the condition of the proband in family H (Figure 8) who did not express the fragile X cytogenetically, but a mentally retarded PM carrier; who also suffers from ambiguous external genitalia (unpublis-

ed observation). Non-expressing males do not express the fragile site, they transmit the mutation to their daughters who are also none expressing for the mutation but can have affected sons. This pattern follows through in the pedigrees where the inheritance of the fragile-X chromosome descended from maternal grandfathers. Apparently males do not inherit any X chromosome pa



**Figure 7.** Family G with a male propositus who had a carrier mother and a normal non-transmitting father.



**Figure 8.** Family H with a male propositus who had a carrier mother and a normal non-transmitting father. There is a preponderance of carriers.

ternally and as such will not be expected to be carriers if their mothers carry normal alleles.

The occurrence of mosaicism in female subjects in this survey is not unexpected. Mosaicism is correlated with high functioning and since FXS is a sex linked condition, mosaicism when it occurs, show a strong pattern.

The pattern of the fragile X inheritance and the behaviour of the alleles will suggest pertinence to genetic counselling particularly in families with high female PM frequencies. Further work with larger sample size and pedigrees with large kindred should prove useful in extending the reliability of our indices for risk evaluation in

the general populations.

**ACKNOWLEDGEMENT**

Thanks to Dr. Ted Brown of the Institute for childhood disabilities, Staten Island NY, for the provision of the base reference materials used at the planning stage of this work.

**REFERENCES**

Allingham-Hawkins DJ, Babul-Hirji R, Chitayat D, Holden JJ, Yang KT,



- Lee C, Hudson R, Gorwill H, Nolin SL, Glicksman A, Jenkins EC, Brown WT, Howard-Peebles PN, Becchi C, Cummings E, Fallon L, Seitz S, Black SH, Vianna-Morgante AM, Costa SS, Otto PA, Mingroni-Netto RC, Murray A, Webb J, Vieri F (1999). Fragile X premutation is a significant risk factor for premature ovarian failure: the International Collaborative POF in Fragile X study--preliminary data. *Am. J. Med. Genet.* 83: 322-325.
- Arinami T, Kondo I, Nakajima S, Hamaguchi H (1987). Frequency of the fragile X syndrome in institutionalized mentally retarded females in Japan. *Hum. Genet.* 76(4): 344-347.
- Belmonte MK, Allen G, Beckel-Mitchener A, Boulanger LM, Carper RA, Webb SJ (2004). Autism and abnormal development of brain connectivity. *J. Neurosci.* 24(42): 9228-9231.
- Blanc DS, D'Angelo J, Lecavelier des Etangs N (1993). (Autism and children with Fragile X syndrome). *Ann. Pediatr. (Paris)* 40(9): 565-572.
- Blomquist HK, Gustavson KH, Holmgren G, Nordenson I, Palsson-Strae U (1983). Fragile X syndrome in mildly mentally retarded children in a Northern Swedish county. A prevalence study. *Clin. Genet.* 24(6): 393-398.
- Brown WT (1995). Perspectives and molecular diagnosis of the fragile X syndrome. *Clin. Lab. Med.* 15(4): 859-875.
- Brown WT, Mezzacappa PM, Jenkins EC (1981). Screening for fragile X syndrome by testicular size measurement. *Lancet* 2(8254):1055.
- Devys D, Biancalana V, Rousseau F, Boue J, Mandel JL, Oberle I (1992). Analysis of full fragile X mutations in fetal tissues and monozygotic twins indicate that abnormal methylation and somatic heterogeneity are established early in development. *Am. J. Med. Genet.* 43(1-2): 208-216.
- Dewald GW, Buckley DD, Surbeck JL, Jalal SM (1992). Cytogenetic guidelines for fragile X studies tested in routine practice. *Am. J. Med. Genet.* 44(6):816-821.
- Fryns JP, Kleczkowska A, Kubien E, Van den Berghe H (1984). Cytogenetic findings in moderate and severe mental retardation. A study of an institutionalized population of 1991 patients. *Acta Paediatr. Scand. Suppl.* 313: 1-23.
- Hinds HL, Ashley CT, Sutcliffe JS, Nelson DL, Warren ST, Housman DE, Schalling M (1993). Tissue specific expression of FMR-1 provides evidence for a functional role in fragile X syndrome. *Nat. Genet.* 3(1): 36-43.
- Howard-Peebles PN, Finley WH (1983). Screening of mentally retarded males for macro-orchidism and the fragile X chromosome. *Am. J. Med. Genet.* 15(4): 631-5.
- Jacobs PA, Mayer M, Abruzzo MA (1986). Studies of the fragile (X) syndrome in populations of mentally retarded individuals in Hawaii. *Am. J. Med. Genet.* 23(1-2):567-572.
- Kahkonen M, Leisti J, Thoden CJ, Autio S (1986). Frequency of rare fragile sites among mentally subnormal schoolchildren. *Clin. Genet.* 30(3):234-238.
- Pembrey M, Baraitser M (1986). Recently recognized chromosomal defects of clinical importance. *Postgrad. Med. J.* 62(724): 131-142.
- Pieretti M, Zhang FP, Fu YH, Warren ST, Oostra BA, Caskey CT, Nelson DL (1991). Absence of expression of the FMR-1 gene in fragile X syndrome. *Cell* 66(4):817-822.
- Pollard-Knight D SA, Akhavan H, Brady MA (1990). Non-radioactive DNA deletion on southern blots by enzymatically triggered chemiluminescence. *Ann. Biochem.* 185: 353-358.
- Primrose DA, el-Matmati R, Boyd E, Gosden C, Newton M (1986). Prevalence of the fragile X syndrome in an institution for the mentally handicapped. *Br. J. Psychiatry* 148: 655-657.
- Sherman SL, Jacobs PA, Morton NE, Froster-Iskenius U, Howard-Peebles PN, Nielsen KB, Partington MW, Sutherland GR, Turner G (1985). Unaffected carrier males in families with fragile X syndrome. *Am J Hum Genet* 37 (5): 956-964.
- Shiue CN, Lin YH, Kuan LC, Lii LM, Tsai WH, Chen YJ, Lin SJ, Kuo PL (2004). Cytogenetic surveillance of mentally-retarded school children in southern Taiwan. *J. Formos Med. Assoc.* 103(3): 218-224.
- Strauss W (1988). Preparation of genomic DNA from mammalian tissue. In: Ausubel FM BR, Sutherland GR, Baker E, Fratini A (1985). Excess thymidine induces folate sensitive fragile sites. *Am. J. Med. Genet.* 22(2): 433-443.
- Thake A, Todd J, Webb T, Bunday S (1987). Children with the fragile X chromosome at schools for the mildly mentally retarded. *Dev. Med. Child. Neurol.* 29(6): 711-719.
- Trnovski J (1992). Semi-dry electroblotting of DNA and RNA from agarose and polyacrilamide gels. *Biotechniques* 13:800-803.
- Van Esch H, Dom R, Bex D, Salden I, Caeckebeke J, Wibail A, Borghgraef M, Legius E, Fryns JP, Matthijs G (2005). Screening for FMR-1 premutations in 122 older Flemish males presenting with ataxia. *Eur. J. Hum. Genet.* 13(1): 121-123.
- Venter PA, Coetzee DJ, Badenhorst A, Marx MP, Op't Hof J, Behari D, Wilmot J, Battson SA (1984). A confirmed prenatal diagnosis of a female fetus with the fragile X chromosome. *Prenat. Diagn.* 4(6): 473-474.
- Venter PA, Gericke GS, Dawson B, Op't Hof J (1981). A marker X chromosome associated with non-specific male mental retardation. The first South African cases. *S. Afr. Med. J.* 60(21): 807-811.
- Walsh P, Erlich HA, Higuchi R (1992). Preferential PCR amplification of alleles: Mechanisms and solutions. *PCR Methods Appl.* 1: 241-250.
- Wohrle D, Hennig I, Vogel W, Steinbach P (1993). Mitotic stability of fragile X mutations in differentiated cells indicates early post-conceptual trinucleotide repeat expansion. *Nat. Genet.* 4(2): 140-142.