FRAGILE X SYNDROME FULL MUTATION FEMALES AT INCREASED RISK FOR MOSAIC TURNER SYNDROME: FRAGILE X LEADS TO CHROMOSOME LOSS

Author:
Daniel DeMizio

Faculty Sponsors:
W.S. Klug
Department of Biology

ABSTRACT

Fragile X Syndrome, the most common form of inherited mental retardation, is characterized by autistic behavior and mild to severe learning disabilities. The disease is caused by the expansion of a trinucleotide repeat located on the *FMR1* (*Fragile X Mental Retardation*) gene located on the X-chromosome. Since the early 1990s, 348 prenatal female samples have been analyzed for Fragile X Syndrome at the NYS Institute of Basic Research in Developmental Disabilities. Of the 94 Fragile X affected females found, five of these cases were also determined to be afflicted with an extremely rare (1 in 20,000-30,000) disease, known as mosaic Turner Syndrome. Five cases of Fragile X/Mosaic Turner affected females discovered in such a small sample size contradict the diseases' low prevalence. This led to the hypothesis that there is a connection between Fragile X Syndrome and Turner Syndrome. In order to strengthen this hypothesis, we assessed the origin of the lost chromosome, and aimed to provide a mechanism by which the chromosome is lost. We believed the maternal Fragile X chromosome was being lost in these cases. Using the *DXS451* and androgen receptor polymorphisms in polymerase chain reactions (PCR) and capillary electrophoresis, analysis of the five affected females and their parents allowed us to size the alleles and to identify the origin of parental chromosome loss. Two long term lymphoblastoid (LTL) cell lines of different full mutation females were also harvested for observations of chromosome loss over time. After allele sizing, 4 of the 5 Fragile X/mosaic Turner cases and 1 of the 2 LTL cell lines in our study depicted loss of the mutated maternal chromosome. These results suggest that the LTL cell lines can be used as a reliable technique for modeling parental sex chromosome loss over time. More importantly, however, these results suggest that a Fragile X chromosome may have a predisposition to be lost in somatic cells.

INTRODUCTION

Fragile X Syndrome is the most common form of inherited mental retardation and accounts for approximately 1.5 in 10,000 of all cases of mental retardation, about 1 in 4,000 for males, and 1 in 8000 for females (Karunasagar *et al.* 2005). The *Fragile X Mental Retardation-1* gene (*FMR1*) was identified in 1991 at the Xq27.3 region of the X chromosome. The disorder is characterized by the expansion of a CGG trinucleotide repeat located in the promoter region of the *FMR1* gene (Fu *et al.* 1991; Oberlé *et al.* 1991; Verkek *et al.* 1991). Those affected with Fragile X Syndrome typically exhibit characteristics such as large ears, prominent jaws, joint laxity, hyperactivity, macroorchidism, attention deficits, autistic behavior, and mild to severe mental retardation.

The disorder itself is caused by the expansion of the CGG trinucleotide repeat to an allele of >200 repeats, and for reasons not yet fully understood, the full mutation is only inherited through maternal transmission (Dobkin 1999). The presence of >200 repeats causes the promoter region of the *FMR1* gene to become highly unstable, resulting in extensive methylation that occurs very early in development. This methylation is the underlying cause of the visible "fragile site," depicted in Figure 1. The existence of this

highly methylated fragile site inhibits the translation of the FMR1 protein (FMRP). This mRNA binding protein is necessary for proper neuronal development (Pieretti *et al*. 1991).

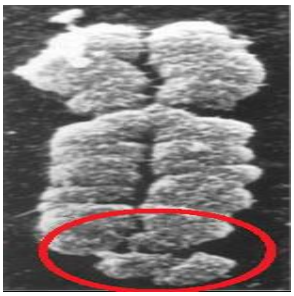

Figure 1.
A Fragile X-chromosome. The red circle contains the region of the X chromosome known as the "fragile site" – an area of extensive methylation caused by a >200 CGG trinucleotide repeat.

Prior to the identification of this *FMR1* gene and the existence of a triplet repeat region, Fragile X Syndrome could only be diagnosed through cytogenetic analysis. Since then, however, several diagnostic methods such as Southern blot, polymerase chain reaction (PCR), and immunhistochemical analyses have been developed (Oostra *et al.* 2001). Current diagnosis for Fragile X Syndrome is largely based on PCR and/or Southern blot analysis of the triplet repeat length in genomic DNA from blood samples, chorionic villi, or amniocyte fluid. These triplet repeat alleles can be separated into three major classes based on size and relative stability: normal, premutation, and full mutation. Alleles with approximately 6 to 60 triplet repeats are considered normal alleles that are stable development and transmission. Alleles with approximately 60 to 200 triplet repeats are considered premutation alleles and are functional in somatic development, yet are often known to expand to the highly unstable full mutation (>200 triplet repeats) upon inheritance (Dobkin 1999).

PCR and Southern analysis are the primary diagnostic methods used at the Institute of Basic Research for Developmental Disabilities. Over a period of nearly 20 years, 94 prenatal females have been diagnosed with the Fragile X full mutation. Throughout this process, five of these 94 cases were also diagnosed with an extremely rare disease known as mosaic Turner syndrome. While Turner syndrome (45,X) is a fairly common disease exhibiting a prevalence of approximately 1 in 3,000 females, mosaic Turner syndrome (45,X/46,XX) is estimated to have a prevalence of 1 in 20,000-30,000 (Sybert *et al.* 2004). These two related genetic disorders result in monosomy X in somatic cells, the loss of an X chromosome. While all somatic cells are affected by the monosomy in Turner Syndrome (45,X), mosaic Turner syndrome results in a mixture of normal somatic cells (46,XX) and the abnormal 45,X. While the exact cause of chromosome loss is not fully understood, approximately 75% of Turner cases have resulted from the loss of a paternal X chromosome, perhaps from nondisjunction and the increased occurrence of X chromosome abnormalities in sperm (Uematsu *et al.* 2002).

The discovery of these five cases of Fragile X and Mosaic Turner affected females found in such a small sample size gave rise to the hypothesis of a causal connection between the two genetic disorders. While cases of mosaic Turner syndrome in Fragile X females had been previously reported (Tejada *et al.* 1994; Shapiro *et al*, 1994; Wilkin *et al*, 2000), no such connection between Fragile X and mosaic Turner syndrome had been made. This study was conducted in order to assess such a connection and provide a possible mechanism for chromosome loss. We hypothesized that the existence of a maternal Fragile X chromosome increases its tendency towards loss during development.

PCR and capillary electrophoresis using *DXS451* and androgen receptor loci allowed us to analyze the genomic DNA five affected females and their parents. We were able to size the alleles and ultimately identify the origin of parental chromosome loss. We were able to use similar methods to analyze two Fragile X female long term lymphoblastoid (LTL) cell lines cultured over a period of 7 months in an attempt to model chromosome loss over time.

MATERIALS AND METHODS

Sample Preparation

Fetal DNA for Southern and PCR analyses was isolated from cultured chorionic villus samples or from cultured amniocytes sent to the Institute of Basic Research from various facilities across the United States. Parental DNA was extracted from blood leukocytes, stored at 4˚C, and isolated using Qiagen: DNEasy Kit materials and protocol. Each DNA pellet (1.5 x 10^4 cells) was resuspended in 200µl PBS, 200 µl AL, 20 µl (1.25mL) Protease K, and incubated for 10 minutes at 70˚C. 200 µl of ethanol were then added and the samples were centrifuged in DNEasy spin column test at 8000rpm for three 1 minute intervals and eluted. Long term lymphoblastoid (LTL) samples were isolated from blood leukocytes, resuspended in 1 ml RPMI containing 2 µg CSA/ml, and incubated in a round bottom tube at 37°C with 5% CO_2. LTL cell lines were harvested and propagated over a period of 7 months. Both the prepared DNA samples and the LTL cell lines were used for Fragile X screening and allele sizing.

Fragile X Screening

Diagnosis for the genetic disorder primarily depends on the triplet repeat length of the *FMR1* gene. At the Institute of Basic Research in Developmental Disabilities, 348 prenatal females have been tested for Fragile X through use of polymerase chain reaction (PCR) and Southern blot analysis of the Fragile X CGG repeat locus. Both techniques are viable methods for comparing genomic restriction fragment patterns produced by sample DNA under gel electrophoresis. The ability for PCR to be applied to samples of ≤ 5 x 10^4 cells, compared to the ≥ 10^6 cells necessary for Southern blot, made it a more convenient technique for amplifying the relatively small prenatal female samples used in this study. Polymerase chain reactions take smaller-sized samples of DNA and create numerous copies of specific regions of genomic information using complementary primers when placed under certain temperature conditions. These amplified regions can then be analyzed by gel electrophoresis, which relies on electric current to separate DNA fragments based on size.

The trinucleotide *FMR1* repeat region was amplified by PCR after digesting each sample DNA with 100 U of *Eco*RI and 50 U of *Eag I*. 100µl PCR reactions were created containing 10mM Tris-HCl, pH 8.3, 50mM KCl, 200 µM dNTP, 2.5U of Ampli*Taq* polymerase,4 mM $MgCl_2$, 1 µM 144F primer (CGCTAGCAGGGCTGAAGAGAAGATG), and 1 µM 632R primer (CTCCTCCACAACTACCCACACGAC). PCR conditions were: 94˚C for 2 min; 23 cycles of 94˚C for 1min; 60˚C for 1min; 72˚C for 2min; and 72˚C for 10min (Dobkin *et al* AJMG 1999). 20 µl PCR products were electrophoresed on a 2% agarose gel in TAE buffer.

Cytogenetic analyses were also performed by clinical facilities at NY Presbyterian Hospital, Columbia University Medical Center, Washington University School of Medicine, and Genzyme Genetics.

A compilation of mosaic Turner/ Fragile X samples were found via these three methods and then analyzed for possible determination of the parental origin of chromosome loss.

Allele Sizing

PCR analysis of the trinucleotide androgen receptor polymorphism and the dinucleotide polymorphism *DXS451* were employed to identify parental origin of chromosome loss in the 5 Fragile X/mosaic Turner cases. 20µl PCR reactions were created containing 100 mM Tris-HCl, pH 8.3, 500 mM KCl, 10mM dNTP, 25mM $MgCl_2$, 0.025U Red*Taq* polymerase, 20µM forward, and 20µM reverse primer. Androgen receptor (*AR*) locus analysis forward and reverse primers used were, respectively, (5' to 3'): TCCAGAATCTGTTCCAGAGCGTGC and GCTGTGAAGGTTGCTGTTCCTCAT. The *DXS451* locus analysis forward and reverse primers used were, respectively, CTTGATCTTCTGAGGAGTGG and TTATTCCTAGGCTTAGGATTC. PCR conditions were 95˚C for 3 min; 35 cycles of 95˚C for 20sec; 64˚C for 20sec; 72˚C for 30sec; and 72˚C for 7min (Uematsu *et al* 2002). The PCR products were run on 3% agarose gel in TAE buffer for gel electrophoresis. The samples were then analyzed by capillary electrophoresis using CEQ 8000 DNA Analysis Systems. This technique relies on an electric field to separate charged species based on charge-to-mass ratios, and in the case of this study, by the varying degrees of certain dye strengths. These ratios made it possible to create graphs displaying *DXS451* and

AR allele sizes. Capillary electrophoresis of 43 µl SLS, 0.8 µl CEQ-Standard 600, and 2 µl of PCR product, permitted for allele sizing of the *DXS451* and *AR* loci for the controls, affected females, parental samples, and LTL cell lines.

LTL cell lines from periodic harvestings over a period of 7 months were subjected to the aforementioned conditions, but, because of limited time, only the *AR* polymorphism was used.

RESULTS

Fragile X Screening

Since 1991, the Institute of Basic Research has performed 693 prenatal diagnoses for Fragile X syndrome with PCR, Southern blot, and cytogenetic analyses. Subjects were selected based on referrals by genetic counselors from a variety of institutions such as Columbia University's New York Presbyterian Hospital, Genzyme Genetics, and Cornell University. Samples largely came from areas in the Northeast of the United States, especially New York. 348 of the 693 samples examined were prenatal females – of which 94 were found to carry a Fragile X full mutation. The criterion of the presence of >200 copies of the trinucleotide CGG was utilized. Five of 94 Fragile X affected prenatal females were also diagnosed with mosaic 45,X/46,XX Turner syndrome. These results are shown in Table 1.

Table 1. Fragile X Screening of Prenatal Females

CGG Repeat size[1]	Females	
	46,XX	45,X/46,XX
<200	254	0
>200	94	5
	348	**5**

[1] CGG Repeat sizes >200 depict Fragile X affected samples.

Analysis of these numbers suggested a correlation between the two genetic disorders, as such a high degree of frequency is unlikely to have occurred by chance. Upon Chi-square analysis, a p-value < 0.001 was calculated, supporting this conclusion. It can, therefore, be inferred that there is a correlation between Fragile X syndrome and the mosaic loss of an X chromosome in developing cells. The existence of a maternally derived Fragile X chromosome in these prenatal females led us to question if it was this mutated maternal chromosome that was lost.

Paternal Origin of Chromosome Loss

Two polymorphisms were studied as a means to compare the relative number of X chromosomes from each parent that existed in the prenatal sample. The two polymorphisms used in this study were the dinucleotide *DXS451* polymorphism and the trinucleotide *AR* polymorphism. In order to conduct this study, a control of 12 unaffected (non-Fragile X/non-mosaic Turner) prenatal females were first analyzed to determine the variance of allele ratios expected in prenatal females where no chromosome loss is expected (Figure 2). Figure 2A represents the allele sizes and signal strengths of the maternal X chromosome expected of a control sample. Figure 2B represents the size and signal strength of a mosaic Turner/Fragile X female. Signal strength, once integrated, represents the relative amount of an allele existing in the PCR product for that sample.

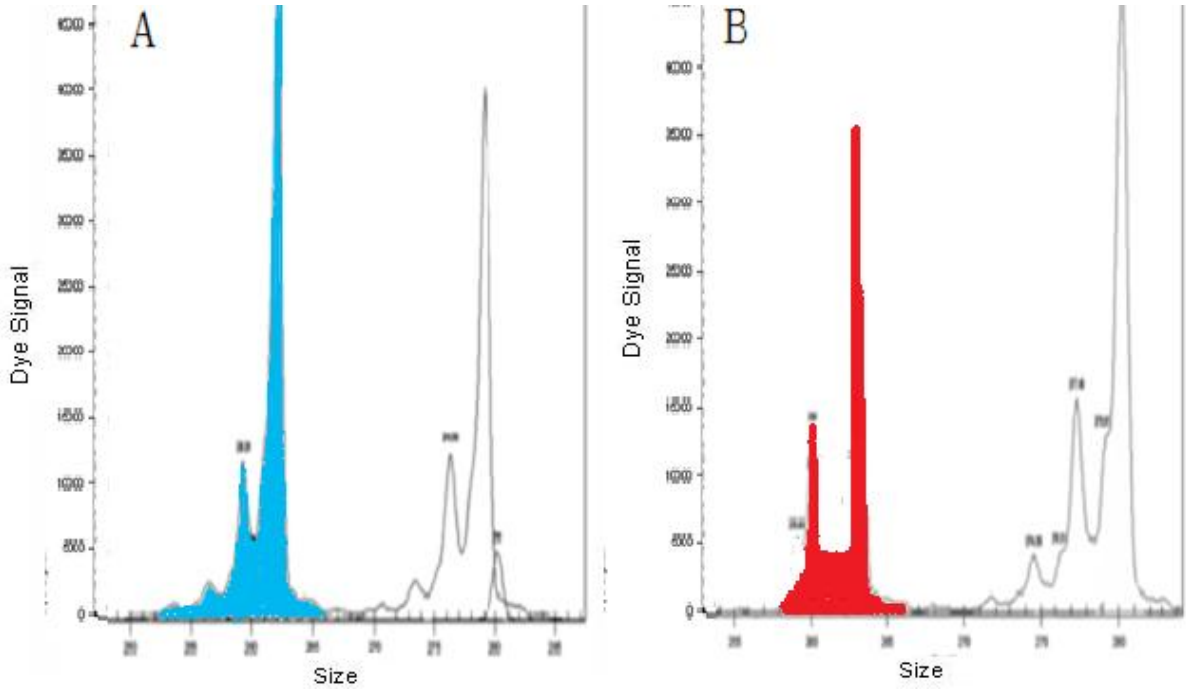

Figure 2.

A comparison of *AR* locus allele ratios, obtained through capillary electrophoresis from *(A)* a control female (in blue) to *(B)* a mosaic Turner/Fragile X female (in red). The X-axis (size) represents length of allele in base pairs, and the Y-axis (Dye Signal) represents the amount of PCR product based on fluorescence in capillary electrophoresis. Graphics were obtained via CEQ 8000 DNA Analysis System.

Mean ratios of maternal to paternal alleles at the *AR* and *DXS451* loci were 1.135 ± 0.113 and 1.671 ± 0.284, respectively. Once this control ratio had been established, a measurement of each parent's allele size was obtained through PCR and capillary electrophoresis. Then, the relative amount of each parent's allele could be measured by taking a ratio of the alleles present in the prenatal samples' PCR product, quantified by integrating the peaks of a particular allele sized by capillary electrophoresis. The detection of chromosome loss could therefore be identified as PCR results with allele ratios that differed by at least 2 standard deviations from the mean value of the control samples. Results obtained through capillary electrophoresis of each individual mosaic Turner case appear in Table 2.

Table 2. Allele Size Ratios for *AR* and *DXS451* Polymorphisms

	AR		*DXS 451*		Cyto	Lost
Case	Ratio	Pvalue	Ratio	Pvalue	%Turner	
1	0.730	**<0.001**	2.339	**0.009**	50	Mat
2	0.879	**0.012**	1.216	0.055	60	Mat
3	0.841	**0.005**	1.573	0.365	14	Mat
4	1.142	0.524	1.202	**0.049**	7	ns[1]
5	0.868	**<0.001**	0.002	**<0.001**	15	Mat
controls	1.135 ± 0.113		1.671 ± 0.284			

[1] not significantly different from the mean.

Only Case 4 did not meet the necessary condition of differing from the mean by at least 2 standard deviations, and was therefore considered inconclusive. In all remaining cases, however, a decrease in the allele ratio (maternal:paternal) represented a loss in the X chromosome. Because the sizes

of parental alleles present in each of the mosaic Turner samples had been sized and identified, the change in the relative amounts of maternal to paternal chromosome also represented which chromosome had been lost. In each case, a decrease in the ratio was observed, representing loss in the maternal chromosome.

LTL Chromosome Loss

In order to find a mechanism by which chromosomes are lost, an easily studied model must be elucidated. Two long term lymphoblastoid cell lines (LTL) of Fragile X females were cultured and propagated for a period of 7 months. PCR analysis of the *AR* locus polymorphism was used on both cell lines to see whether chromosome loss over time can be seen in non-Turner cases and if it could be used as a model for such loss. Allele ratios (maternal:paternal) for both cell lines were examined for eight intervals over the 7 month propagation period (Figure 3). Only 1 of the 2 cases demonstrated any significant change in maternal to paternal allele ratio, often differing more than 4 standard deviations from the expected *AR* locus ratio. Case 1 showed a decrease in the ratio of maternal to paternal allele amounts, from 1.31 to 0.78. Case 2, on the other hand, showed no significant change with an allele ratio at a fairly constant ratio of 1.34. Although further testing must be done, this seems to suggest that some of the cultured cells in Case 1 had experienced partial loss of a chromosome over time. Using such LTL cell lines as models, it may be possible to study the reasons why the Fragile X chromosome is prone to loss.

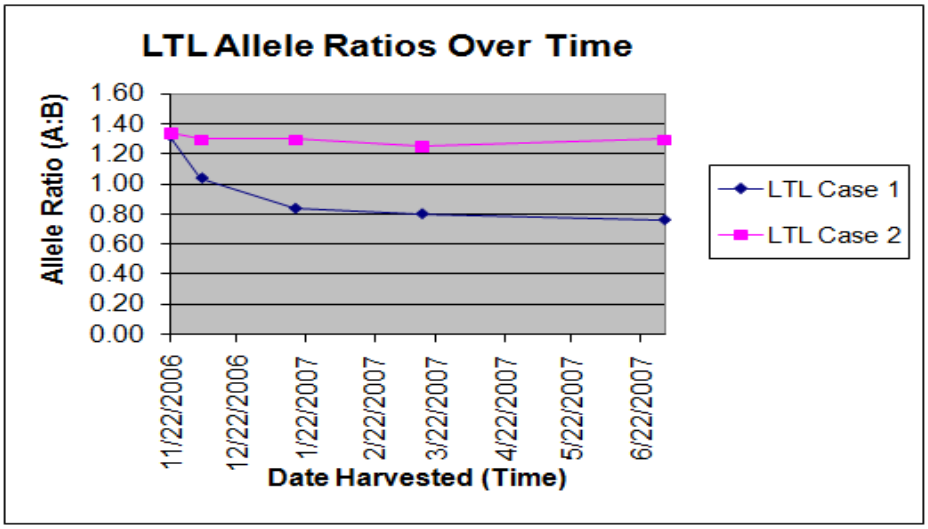

Figure 3.
Comparison of LTL Allele Ratios (Maternal to Paternal) over a period of 7 months (n = 2). Case 1 showed loss of the maternal chromosome over time, whereas case 2 showed no significant change.

DISCUSSION

The transmission mechanism of expression and the overall molecular basis for Fragile X Syndrome are not yet fully understood. Its similarities to autism, however, recently have caused this genetic disorder to become a prime focus of research. The gender of the carrier parent, gender of offspring, and the number of CGG repeats are all important factors that influence the expression of Fragile X Syndrome (Karunasagar *et al*. 2005). The ability of normal women to transmit the fragile X despite no manifestation of any phenotype, as well as the tendency of female premutation (repeats in 60-200 range) alleles to expand during transmission, has made screening for Fragile X Syndrome an essential, yet difficult task (Nolin *et al.* 2003). The Institute of Basic Research is one such provider of diagnostic screening for Fragile X Syndrome, as well as a monitor of families in danger of premutation allele expansion. It is estimated that 1 in 4,000 women carries a full mutation allele, and 1 in 250 carries a premutation allele capable of expansion to a full mutation (Crawford *et al.*, 2001).

At the Institute of Basic Research, screening for the disorder is primarily accomplished through the determination of the CGG triplet repeat length found in the *FMR1* gene. While PCR is the preferred method of diagnosis because of its speed, accuracy, and effectiveness, samples with very large repeats often require standard EcoRI-EagI Southern blot analysis (Dobkin 1999). Figure 4 depicts the ways in which both methods provide reliable diagnostic patterns that enable technicians to determine Fragile X status.

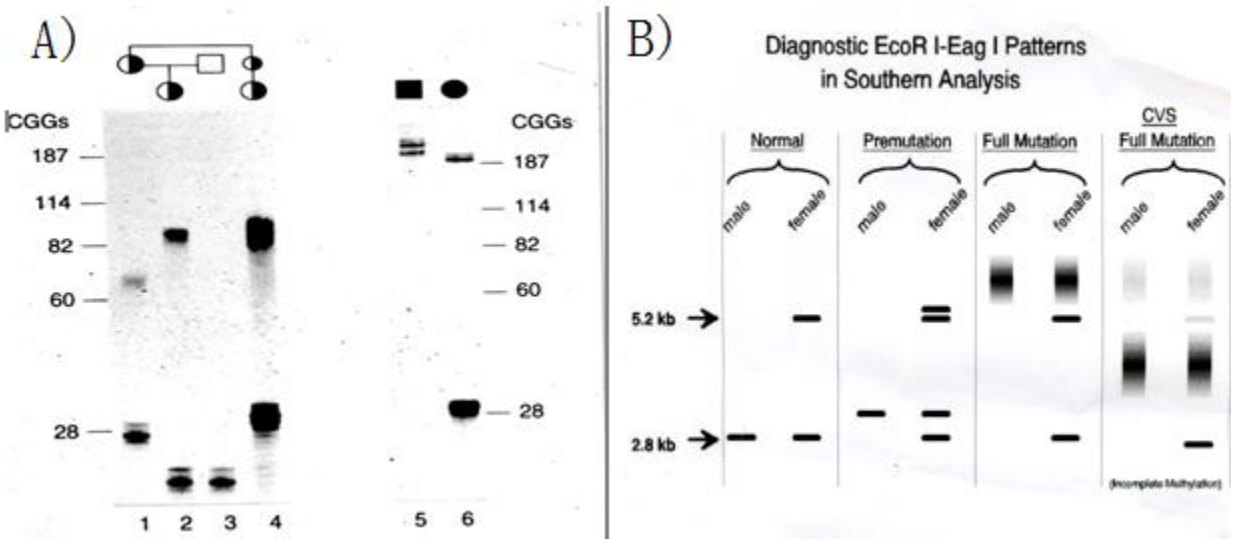

Figure 4.

A). Diagnostic patterns for PCR based detection for Fragile X Syndrome based on the number of CGG repeats. Repeat sizes in the >200 range (Lanes 5 and 6) indicate Fragile X status. B) Diagnostic patterns for Southern analysis for detection of Fragile X Syndrome. Digestion of fetal or chorionic villi (CVS) genomic DNA by EcoRI - Eag I yield products with varying fragment sizes. These commonly found patterns and sizes represent the normal, premutation, or full mutation alleles caused by the selective digestion by the two endonucleases.

In testing for Fragile X Syndrome, mosaic Turner syndrome was also detected in 5% of females carrying the full mutation. No other mosaic Turner syndrome cases were found in the remaining normal or premutation prenatal females. In light of the low frequency of mosaic Turner syndrome in the general population (1 in 20,000-30,000), the discovery of 5 cases in a sample size of 348 prenatal females was very unusual. While three cases of mosaic Turner syndrome in Fragile X full mutation females were previously reported (see below), an increased prevalence of mosaic Turner syndrome related to Fragile X Syndrome had never been suggested. These three published studies, summarized in Table 3, depict the samples' age, percent of cells found to be 45,X, origin of chromosome loss, and method of identification. Identification of the lost chromosome was determined either by diminished signal strength from maternal X chromosome RFLP markers in Southern analysis (Tejada *et al.* 1994), or by cytogenetic observation of the Xq23.7 fragile site in 45,X and 46,XX cells (Shapiro *et al.*1994; Wilkin *et al.* 2000). As Table 3 shows, 2 of 3 published cases of mosaic Turner/Fragile X full mutation females were caused by loss of a maternal X chromosome.

Table 3. Previously Recorded Fragile X Mosaic Turner Samples

Reference	% 45,X	age	lost X identified by	X Loss
Tejada et al.	84	adult	RFLP markers in Southern Analysis	Mat

Shapiro et al.	74	adult (18 yr)	Xq27.3 Fragile Site	Mat
Wilkin et al.	38	fetus (10 wk)	Xq27.3 Fragile Site	Pat

These findings led us to ask whether the 5 cases of mosaic Turner/Fragile X prenatal females also resulted from loss of the maternally derived Fragile X chromosome. To test this hypothesis, we used PCR analysis of the dinucleotide polymorphism *DXS451* and the trinucleotide polymorphism at the *AR* locus to quantify the X chromosomes and identify the origin of loss. We chose these 2 loci because they are relatively close to the *FMR1* gene and are therefore not prone to be separated by crossing over. It was important to minimize the possibility of crossovers so that we could accurately ascribe allele sizes determined through capillary electrophoresis to a specific parent. Similarly, these two polymorphisms have different ranges for allele sizes. The polymorphisms therefore provided a means for ensuring the validity of our results. The *DXS451* locus yields a PCR product of roughly 182-194 base pairs, whereas the *AR* locus yields products of roughly 255-267 base pairs.

Of the 5 cases found at the Institute of Basic Research, 4 out of 5 were determined to have met our criterion for detectable chromosome loss by differing from control allele ratios by at least 2 standard deviations. To determine these ratios, the parents of the 5 samples were run under PCR for both the *AR* and *DXS451* loci and then sized via capillary electrophoresis. Sizing of both parental alleles allowed us to determine the origin of the alleles found in the full mutation females. Once the identity of these alleles was ascertained, the amount of each allele could be quantified by finding the area under each peak. The ratio of maternal to paternal allele could then be compared to the controls. This ratio decreased in the 4 significant cases, differing by at least 2 standard deviations from the mean. This decrease in allele ratio (maternal:paternal) represents loss of the maternal, Fragile X chromosome. Based on these results, as well as the previously published results, one can infer that the Fragile X chromosome is predisposed to loss during development.

The mechanism by which the chromosome is lost and why this predisposition occurs is not fully understood at this time and has become the focus of further research. It is hypothesized that delayed replication, chromatin condensation, and double strand breaks associated with the fragile site influence the occurrences of chromosome loss (Wang 2006). Future studies, however, require a means by which chromosome loss can be studied. Therefore, more LTL cell lines will need to be examined over long periods of propagation as models to observe the tendency of Fragile X chromosomes to be lost over time.

ACKNOWLEDGEMENTS

Special thanks to the staff of the Human Genetics Department at the Institute of Basic Research in Developmental Disabilities, especially: Dr. Carl Dobkin, Dr. Sally Nolin, Dr. Ted Brown, Xiao-hua Ding, and Nicole Hosmer. I also thank Professor W.S. Klug, who coordinates the Biology Research Internship (BIO399) program at TCNJ, For his guidance, input, and support.

REFERENCES

Crawford, D.C., Acuna, J.M. & Sherman, S.L. (2001) FMR1 and Fragile X syndrome: Human genome epidemiology review. *Am. J. Med. Genet.* 3:359-371.

Dobkin, C., Nolin, S., and Brown, W.T. (1999) Accelerated prenatal Diagnosis of Fragile X Syndrome by Polymerase Chain Reaction Restriction Fragment. *Am. J. Med. Genet*. 83:338-341.

Fu, Y.H., Kuhl, D.P., Pizzuti, A., Pieretti, M., Sutcliffe, J.S., and Richards, S. (1991) Variation of the CGG repeat at the fragile X site results in genetic instability: resolution of the Sherman paradox. *Cell*; 67:1047-1058.

Karunasagar, A., Pandit, L., Kumar, S., and Karunasagar, I. (2005) Use of methylation sensitive polymerase chain reaction for detection of fragile X full mutation & carrier state in males. *Indian J. Med. Res.*; 122:429-433.

Nolin, S., Dobkin, C., and Brown, W.T. Molecular analysis of Fragile X Syndrome. In: Current Protocols in Human Genetics. Eds. Dracopoli NC, Haines JL, Korf BR. Wiley New York (2003); 9.5.14.

Oberle, I., Rousseau, F., Heitz, D., Kretz, C., Devys, D., Hanauer, A., and Boue J. *et al*. (1991) Instability of a 550-basepair DNA segment and abnormal methylation in fragile X syndrome. *Science* 252:1097-1102.

Oostra, B.A., and Willemsen, R. (2001) Diagnostic tests for fragile X (FRAXA). *Expert Rev. Mol. Diagn.*; 1: 226-232.

Pieretti, M., Zhang, F.P., Fu, Y.H., Warren, S.T., Oostra, B.A., and Caskey CT. (1991) Absence of expression of the FMR-1 gene in fragile X syndrome. *Cell*; 66:1047-1058.

Shapiro, L.R., Richard, Simensen J., Wilmo, P.L., Fisch, G.S., Vibert, B.K., Fenwick, R.G., Tarleton, J., and Phelan, M.C. (1994) Asymmetry of Methylation With FMR-1 Full Mutation in 2 45,X/46,XX Mosaic Females Associated With Normal Intellect. *Am. J. Med. Genet.* 51:507-508.

Sybert, V.P., McCauley, E. (2004). Turner's syndrome. *New Eng. J. Med.* 351:1227-1238.

Tejada, M.I., Mornet, E., Tizzano, E., Molina, M., Baiget, M., and Boue, A. (1994). Identification by molecular diagnosis of mosaic Turner's syndrome in an obligate carrier female for fragile x syndrome. *Birth Defects Orig. Artic. Ser.* 26(4):209-223.

Uematsu, A., Yorifuji, T., Muroi, J., Kawai, M., Mamada, M., Kaji, M., Yamanaka, C., Momoi, T., and Nakahata, T. (2002) Prenatal origin of normal X chromosomes in Turner syndrome patients with various karyotypes: Implications of the mechanism leading to generation of a 45,X karyotype. *Am. J. Med. Genet.* 111:134-199.

Verkerk, A.J.M.H., *et al.* (1991) Identification of a gene (FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome *Cell*, 65: 905-914.

Wang, Yuh-Hwa. (2006) Chromatin structure of human chromosomal fragile sites. *Cancer Letters* 232:70–78.

Wilkin, H., Tuohy, J., and Theewis, W. (2000) Prenatal diagnosis of fragile X and Turner mosacism in a 12-week fetus. *Prenat. Diagn.* 20:851-856.