The Frequency of Chromosomal Abnormalities in Patients Referred for Fragile X Analysis*

HON FONG LOUIE MARK, Ph.D.,†
JO-ANN BLAYMORE BIER, M.D.,‡
and PATRICIA SCOLA, M.D.¶

†Laboratory of Cytogenetics, FISH, and Genotoxicology,
and ‡Child Development Center,
Rhode Island Hospital

and
Brown University School of Medicine,
Providence, RI 02903

ABSTRACT

The present paper summarizes our existing database on chromosomal abnormalities found in patients referred because of a question of the Fragile X Syndrome during the period from January 1, 1990 to June 30, 1995. Cytogenetic results were derived from testing performed at the cytogenetics laboratory at Rhode Island Hospital. All positive fragile X individuals detected among our sample population represent index patients from separate kindreds. Of a total of 327 cases referred for fragile X testing, 10 (3.06 percent) were found to be positive for fragile X by either cytogenetics alone or by both cytogenetics and DNA testing, 12 (3.60 percent) were found to be positive for either a numerical or structural chromosomal abnormality, while 10 (3.06 percent) were found to exhibit a heteromorphism. Positive chromosomal findings included numerical chromosomal abnormalities of the sex chromosomes and autosomes, deletions, and translocations. Heteromorphism mostly involved an increase in the length of heterochromatic regions of certain chromosomes as well as a pericentric inversion of a chromosome 9, usually considered normal variants. It is concluded that chromosomal abnormalities other than fragile X are found with equal and, in some cases, higher frequency than the frequency of fragile X positivity in patients referred for a question of the Fragile X Syndrome. Our figures, consistent with those reported in the literature, underscore the value of routine karyotyping in this population of patients. Except under special circumstances, it is important that GTG-banding analysis be performed so that the entire human genome be examined in addition to scoring for the fragile X mutation on Xq27.3. Especially in view of the recent finding of the relative rarity of this condition, the exclusive use of DNA analysis is not advised.

* Send reprint requests to: Hon Fong Louie Mark, Ph.D., Director, Laboratory of Cytogenetics, FISH, and Genotoxicology, Rhode Island Hospital, 593 Eddy Street, Providence, RI 02903.
Introduction

The frequency of the Fragile X Syndrome (FXS) has been estimated to be ~1 in 1500 males and from ~1 in 2000 to 1 in 3000 females.\textsuperscript{1,2} It is considered to be one of the major inherited causes of mental retardation in males. The mutation responsible for the fragile X syndrome was identified in May of 1991 by three independent groups using positional cloning strategies (Verkerk et al,\textsuperscript{3} Yu et al,\textsuperscript{4} and Oberle et al\textsuperscript{5}). Patients with the Fragile X Syndrome (figure 1) exhibit the folate-sensitive sites at Xq27.3 as shown by Opitz and Sutherland.\textsuperscript{5} Clinical features usually associated with the Fragile X Syndrome include developmental delay, learning disabilities, mental retardation, autism, avoidance behavior as well as hyperactivity and attention deficit, speech and language delay, unusual hand mannerisms, long and narrow facies with moderately increased head circumference (>50th percentile), prominence of the jaw and forehead with particularly large and mildly dysmorphic ears, hyperextensible joints, high arched palate, pes planus, pectus excavatum, and mitral valve prolapse. Macro-orchidism is a common finding in post-pubescent affected males.\textsuperscript{2}

While a combination of these features may be unique to the Fragile X Syndrome, any one of the isolated features mentioned is not. For example, some clinical features of the syndrome overlap those associated with low level mosaicism for trisomy 8, such as the long face,

![Figure 1](image_url)

**Figure 1.** The Fragile X Syndrome: cytogenetic manifestation. Arrow points to fragile site at Xq27.3.
prominent forehead, large ears, transverse palmar crease, and mental retardation.\textsuperscript{7,8} It is for this reason that some patients with the mosaic trisomy 8 syndrome and the Klinefelter genotype, for example, were referred for fragile X analysis. In view of this, it was decided to determine the frequency of other chromosomal abnormalities present in patients initially suspected of having the Fragile X Syndrome from our center. The data represent cases analyzed in our laboratory for the past five and a half years, from January 1, 1990 to June 30, 1995.

**Materials and Methods**

**STUDY SUBJECTS**

The subjects of this study were patients referred to the cytogenetics laboratory at Rhode Island Hospital for “fragile X analysis.” The actual reasons for ordering fragile X analysis vary widely. Indications may be listed as “to rule out fragile X”, mental retardation, learning disability, autism, attention deficit disorder, or a number of other similar presentations.

Patients referred for fragile X testing usually originate from the Rhode Island Hospital’s Child Development Center as well as other area pediatricians and/or internists.

**LABORATORY METHODOLOGY**

Peripheral blood samples for chromosome studies were collected in heparinized tubes. Lymphocytes were cultured for 72 hours with phytohemagglutinin using standard techniques.\textsuperscript{9,10,11} Fragile X site expression was induced by one or more standard treatment protocols upon request from the referring physicians. Scoring of other sites besides the fragile X site at Xq27.3 was performed to provide an internal control. In our most frequently used protocol, fluorodeoxyuridine (F UdR, 10 μl of \(10^{-7}\) M final concentration),* methotrexate (MTX, 0.1 ml of \(10^{-5}\) M final concentration),* and Trimethoprim (TMP, 0.1 ml of 13 mg/mL final concentration), were added to each 5 ml of culture 72 hours after the initiation of the culture. Twenty-one hours after the addition of the induction agents, 32 μl of stock Colcemid (10 μg/mL)\textsuperscript{†} were added. Three hours later (or 24 hours after the addition of additives) cells were harvested according to a modification of the methods of Moorhead et al.\textsuperscript{9} The GTG-banding analyses\textsuperscript{12,13} were simultaneously performed to rule out rearrangements.

When fragile X cytogenetic testing was performed, at least 50 percent of the cells scored for fragile Xq27.3 were derived from cultures in which fragile site expression was induced by anti-metabolites (e.g., 5-fluorodeoxyuridine and methotrexate) in accordance with various guidelines, such as those from the Pacific Northwest Regional Genetics Group.\textsuperscript{‡} The presence of the fragile site in putative cases was confirmed by GTG-banding. When a fragile X study was performed, it was considered positive when at least 4 percent of metaphases in the cytogenetic analysis contained a fragile X chromosome. Borderline cases were repeated and/or confirmed by molecular testing. In a cytogenetic study, at least 100 cells were scored. Scoring of other breaks and gaps was used as a means of internal control. Where indicated, testing of deoxyribonucleic acid (DNA) was performed by reference laboratories. In general, when DNA testing was ordered, a routine three-day stimulated culture of peripheral blood was also performed to rule out the presence of constitutional chromosomal abnormalities present elsewhere in the genome.

\* Sigma Chemical Co., St. Louis, MO.
\† Gibco Life Technologies, Inc., Grand Island, NY.
\‡ Personal communications.
The designation of chromosomal abnormalities was in accordance with An International System for Cytogenetic Nomenclature and others.

Results and Discussion

The results from cytogenetic testing of patients referred for fragile X analysis from the period of January 1 of 1990 to June 30 of 1995 follow. All positive fragile X individuals detected among our sample population represent index patients from separate kindreds, as were those exhibiting chromosome heteromorphisms. From a total of 327 specimens accessioned, ten (3.06 percent) were positive for fragile X either by chromosome alone or by both chromosome and DNA. An equal number (3.06 percent) was found to exhibit heteromorphisms, which included an increase in the length of the heterochromatic region of the Y chromosome, an increase in the length of the satellited short arm of chromosome 15, an increase in the length of the heterochromatic region of the long arm of chromosome 9, and a pericentric inversion of chromosome 9, all of which are usually considered normal variants.

In addition, from the sample of 327 subjects, 12 cases (3.60 percent) were detected with numerical and structural chromosomal abnormalities. The former included individuals with numerical sex chromosomal abnormalities, such as XXX, XXY and XYY and individuals mosaic for autosomal abnormalities, such as an extra chromosome 8 and extra unidentifiable markers. The latter included apparent deletions of a chromosome 9 and a chromosome 21, and two translocations: t(3;14) and t(5;15). A recent case of a derivative Y chromosome (from a Y; autosome translocation) was not included in the frequency estimate in this study.

Although these results are understandably lower than the frequency of 6 to 7 percent non-fragile X chromosomal disorders in patients with features of the Fragile X Syndrome derived from surveys of institutions for individuals with retardation previously reported in the literature, it is consistent with previous findings that other chromosomal abnormalities are as frequent, or more frequent, than fragile X positivity in patients referred for “fragile X testing.” The frequency of 3.60 percent (of other chromosomal abnormalities) derived in our center is also necessarily an underestimate. This is because pre-analytic variables, such as test-ordering practices, were not always within our control. Fragile X DNA tests were sometimes processed as stand-alone samples.

Other centers have made similar observations supporting a comparable frequency of other chromosomal abnormalities in patients referred for “fragile X testing”. (See for example, Marini et al.) For example, the overall positive rate for fragile X in one study was found to be 3.5 percent with an incidence of other chromosomal abnormalities of 3.7 percent. These figures were obtained using either cytogenetics alone or a combination of DNA analysis and routine karyotyping. The latter figure, the overall incidence of other chromosomal abnormalities of 3.7 percent, underscores the value of routine karyotyping in this patient population and most likely reflects the phenotypic variability of fragile X and its clinical overlap with other chromosomal abnormalities. Accordingly, the exclusive use of DNA analysis for fragile X in the initial work-up of these patients is not advised.

A widely utilized strategy is to perform conventional cytogenetics testing using GTG-banding in addition to DNA analysis of the FMR-1 locus during the initial stages of testing. This approach is acceptable if testing is performed in accordance with professional standards and guidelines and if the combined cost of the two separate tests is reasonable. While in
some centers the inclusion of DNA testing with initial cytogenetics may not be significantly more costly than that for cytogenetics alone, cost of the two tests in other centers may be prohibitive. In the latter case, it is suggested that the current practices in fragile X testing be re-evaluated to avoid indiscriminate use.

For example, population carrier screening for the FMR1 gene is not recommended at this time except as part of a well-defined clinical research protocol. The nature of the FMR1 mutation and its inheritance are complex, and testing necessitates close-monitoring by appropriately trained individuals, which many centers currently still cannot adequately provide. Thus, in view of the previous information, and of the current climate in healthcare, a thorough review of test ordering practices is recommended. The observation in a report by Morton et al that the fragile X syndrome is even less common than previously estimated (on the order of 1 in 2197) further emphasizes the need to rule out other chromosomal abnormalities besides the fragile site at Xq27.3.

As additional knowledge is gained on this intriguing genetic disease, newer and more efficient protocols will no doubt develop. Until such a time when a more rapid and cost-effective test is developed (e.g., Willemsen et al), fragile X testing should not be performed indiscriminately.

Acknowledgment
Thanks are extended to Kathleen Zolnierz, Robin Kiernan, Dela Rajaratnam, William Campbell, Odete Alves, Elizabeth Hann, and other members of the cytogenetics laboratory for technical and secretarial help. The continued support of Dr. Roger Mark and the dedicated staff of the Laboratory of Cytogenetics, FISH and Genotoxicology at Rhode Island Hospital is acknowledged.

References


