

Advances in knowledge of molecular genetics, cytogenetics and biochemical genetics have led to availability of diagnostic tests for various genetic disorders. The most important application is use of this new technology in prenatal diagnosis for lethal, incurable and chronic disabling genetic diseases. In Utero diagnosis has become a main option for parents who come for genetic counseling. Prenatal diagnosis offers prospective parents the assurance of having an unaffected child, when the risk of having an affected one is high. Clinical genetics is one of the most rapidly advancing fields in medicine. Much of this progress has been propelled by recent advances in molecular genetics. A major contribution of these advances has been in the areas of prevention and avoidance of disease. Genetics cuts through all medical subspecialties and has a pivotal role to play in reproductive health.

In our country it is important to prevent birth of a child with a malformation or a genetic disease thus reducing the socio-economic burden of a handicapped child. Moreover if the normality of a fetus is ensured, it is likely that the parents will limit the number of pregnancies, so that they will more readily accept family planning measures.

Common genetic disorders in Indian population are chromosomal disorders such as Down Syndrome, Thalassaemia, Hemophilia and Duchenne muscular dystrophy. There is no definitive treatment for Down Syndrome and Duchenne muscular dystrophy while the treatment for thalassaemia and hemophilia are very expensive. Fortunately the new genetic technologies provide methods for the prenatal diagnosis of these disorders thus preventing birth of affected children and reducing the burden on the family and the society.

These genetic technologies are extremely cost effective when weighed against the cost of treatment of affected children lasting many years.

Keeping these points in view, research areas include molecular basis of fragile X syndrome with emphasis on identification of premutation carrier females because they have a variable risk for ovarian dysfunction and also are at a high risk of producing affected offspring with fragile X syndrome. The other projects on chromosomal basis of reproductive loss and genetic basis of heart disease have been addressed with emphasis on disability reduction and pregnancy losses. Cytomolecular techniques of Telomeric-FISH (T-FISH) and sperm FISH and comparative genomic hybridisation (CGH) to detect subtle changes in the genome have also been established.

7.1 Molecular Characterization of Fragile X Syndrome

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Project Associates:	Shyla Menon, Rashmi Adhia, Aruna D'souza, H. Gawde and Shiny Babu
Collaborators:	BMC (Brihan Mumbai Municipal Corporation)
Duration:	2000-2007

The fragile X syndrome (FXS) is a frequent cause of inherited mental handicap affecting 1 in 4000 males. The clinical phenotypes consist of large head, prominent forehead, large ears, double row of teeth, prognathism, speech defects and poor eye-to-eye contact. The molecular basis of this disorder is due to the expansion of CGG repeats of >200 times in affected males and between 52-200 in premutation carrier. Premutation carrier can transmit this disorder to their offspring. Hypermethylation of the Fragile X Mental Retardation 1 (FMR1) promoter region silences the FMR1 gene.

The objectives of the study were to i) investigate validity of the analysis of FMR1 protein expression in blood smears as a screening method for FXS, ii) study the known mutations by PCR for CGG expansion, iii) identify new mutations by DNA sequencing.

Using commercially available antibody to FMRP, we standardized a simple rapid cost effective immunocytochemical test. Details of protocol (Annual Report 2000, prepared by GRC) and analysis of 165 cases was documented in the last annual report (Annual Report 2002-03, p 115-117).

Over the last year this project was extended to eighteen special schools of Bombay Municipal Corporation as part of their mental health programme viz Mumbadevi, Parel, Mumbai Central, Worli, Dadar, Sion, Bandra, Khar, Kurla, Kandivli, Goregaon, Borivli, Ghatkopar, Mulund, Bhandup, Santacruz, chembur and Vile Parle.

After informed consent blood smears were collected from 364 cases of idiopathic mental retardates from 18 special schools. In 60 individuals the antibody test was negative for FMRP, suspecting Fragile X syndrome. Blood for DNA was collected and sent to our co-investigator Dr. Thelma B.K., University of Delhi. Only 3 patients were confirmed to have Fragile X Syndrome.

During the year various PCR methods were tried. Because of high GC content in the region these methods were unsuccessful. Now a new set of primers specific for fragile X syndrome and special amplifications system has

been obtained. Also a kit for detection of fragile X syndrome is being obtained for used in the current year.

7.2 Cryptic Chromosomal Rearrangements Amongst Couples with Recurrent Abortions in Indian Population

Principal Investigator: **Zareen Patel**

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Duration: 2002-2007

In the Indian scenario, having a baby is a life changing experience for the couple. Unfortunately one in six pregnancy abort, leaving the couple psychologically and emotionally drained and attaching a social stigma to their lives. Various genetic factors are known to be associated with recurrent spontaneous abortion (RSA) including single gene mutations, polygenic and cytogenetic causes.

The objectives of the study were to i) establish the frequency of cryptic chromosomal abnormalities by karyotyping followed by telomeric FISH carried out on Carl Zeiss Axiopscop II microscope (Fig. 105), ii) study the implication of aneuploidies by sperm FISH as a male contributing factor for RSA

In our previous study of 1000 individuals of Indian origin with 3 or more abortions was included in this study. A standard workup of RSA included Hysterosalpingography, Cardiolipin antibody, parental karyotype and TORCH titres. Chromosomal rearrangement occurred in 18 cases (1.8%). Of this 13 had a structural and 5 had numerical defects. Reciprocal translocations appeared to be predominant in females i.e. 8 out of 9 where as male shows predominants of chromosomal variants. Since chromosomal abnormalities were found in 2 percent cases. The main objectives remaining 98 percent for any cryptic chromosomal rearrangements and to correlate sperm morphology count and aneuploidies for better obstetric managements. Further FISH analysis using telomere specific probes were used to detect cryptic translocation in the chromosomal telomeric region. No cryptic translocation were noted. Further 100 individuals were karyotyped and T-FISH was carried out in 10 couples and 5 normal individuals (Fig. 106A & B). No abnormalities were seen. 43 semen samples (4,30,000 cells) from male partners of the couples with 3 or more abortions were analyzed for aneuploidies for chromosomes 13, 21, 18, X and Y using multicolor LSI (13, 21) and CEP (18, X and Y) FISH probes. FISH analysis showed marked deviation from normal in 5 patients out of the 43 analyzed (11.6%). The mean frequency of disomic sperms (X-X, X-Y, Y-Y, 13-13, 21-21) was

14.65 per 10^4 cells (range 1-190), frequency of total diploid sperms was 10.1 per 10^4 cells (range 1-175) and frequency of total nullisomic sperms was 10.75 per 10^4 cells (range 1-75). Literature survey suggests a 0.3-0.5 percent abnormal cells and so patients showing more than 0.5 percent abnormality were considered to be contributing abnormal gametes which might be major cause for abortions. Details of sperm count and percentage of abnormal forms were also obtained wherever possible and they were analyzed for correlation. It was observed that patients with high percentage of morphological abnormalities for sperm cells had normal FISH results (Fig. 106D, E & F). The analysis revealed no correlation between aneuploidies and sperm morphological abnormalities as well as between aneuploidies and sperm counts. Our study suggests that sperm morphology and count in themselves are not sufficient to rule out the role of sperms in spontaneous abortions. During the current year comparative genomic hybridization will be standardized. Over the next year we will study sperm FISH aneuploidies from carriers of chromosomal rearrangements.

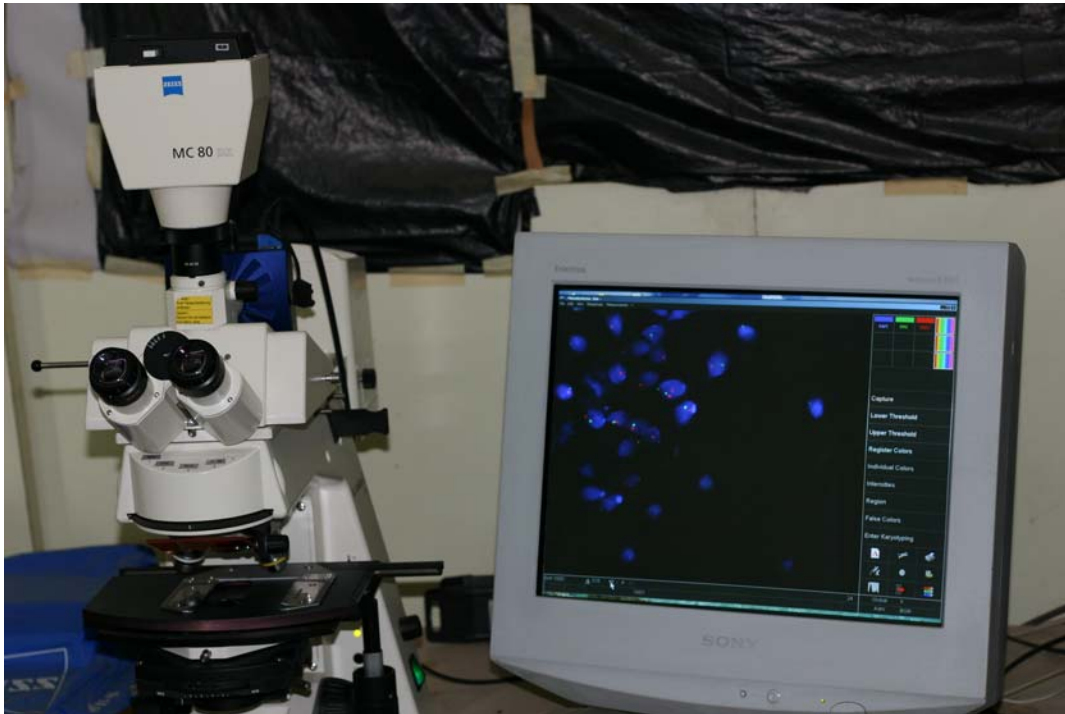


Fig. 105: Carl Zeiss Axioscop II plus with software for karyotype FISH, M-FISH and CGH.

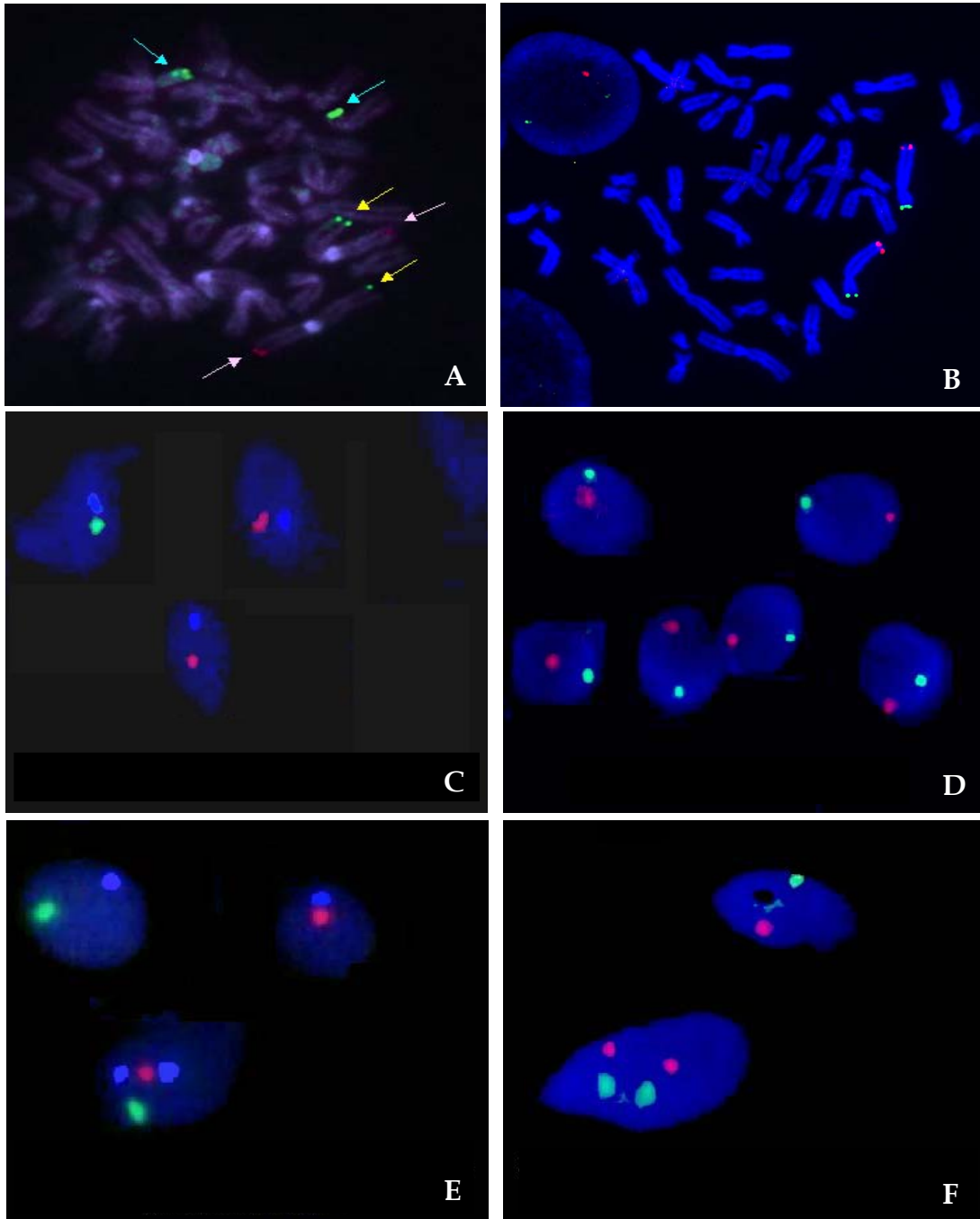


Fig. 106: (A): T-FISH showing signals for 1p, 1q & Xp; (B): T - FISH showing signals for 5p & 5q; (C): Sperms showing signals for normal Ch 18 and Y or Ch 18 and X; (D): Sperms showing signal for Ch 13 and 21 and a diploid cell with two Ch 13 and two Ch 21; (E): Sperms showing signals for Ch 18 and Ch X, Ch 18 and Ch Y and a diploid cell with two Ch 18, Ch X and Ch Y; (F):Sperms showing signals for normal Ch 13 and Ch 21.

7.3 MTHFR 677 C→T as a Genetic Risk Factor for Down Syndrome, Spontaneous Embryo Loss and Neural Tube Defects

Principal Investigator: **Zareen Patel**

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Duration: 2005-2008

Down syndrome or Trisomy 21, where failure of normal segregation during meiosis, is a complex genetic disorder. Except for advanced maternal age, no major risk factors are known. In human the pathogenesis of human spontaneous abortions involves a complex interaction of several genetic and environmental factors. Failure of closure of developing neural tube leads to a number of related conditions collectively termed as neural tube defects. The precise etiology of such conditions is not known, but can involve a number of environmental and genetic factors.

There are many families where one or more of these disorders co-exist. It is also confirmed that 80 percent fetal loss is associated with Down syndrome. The objectives of the study were to evaluate the potential association of MTHFR mutation in families with a child with Down's syndrome, NTD, and spontaneous abortions. This would clarify the mode of inheritance and the mechanisms of gene expression for these disorders. A group of control individuals were studied to find out the frequency of the aberrant alleles in them.

A pilot study was initiated in the patients referred to the Genetic Research Centre. Six nuclear families with history of Down's syndrome and 5 couples with spontaneous abortions were included in the study. For control population, 5 normal fathers, 5 mothers, 5 children were recruited. None of them had any history of any of these disorders. The forward primer used was 5'-TGAAGGAGAAGGTGTCTGCGGGA-3' and the reverse primer was 5'-AGGACGGTTCGGTCAGAGTG-3'.

The PCR product is of 198 bp in size. The palindromic sequence recognized by the restriction endonuclease Hinf I is 5' gantc-3' and the enzyme produces a cut only when there is a polymorphism comprising of a transition of C→T at 677th base pair.

When the polymorphism 677 C→T is present, the recognition sequence is achieved and hence recognized by the enzyme. But in case of absence of polymorphism there is no cut produced and hence one can observe only a single band of DNA of 198 bp on electrophoresis. In the event of presence of

heterozygosity 2 bands of DNA, 198 bp and 175 bp can be observed. When homozygous polymorphism is present only one DNA band is observed, of the length 175 bp. In the subjects where the PCR studies were carried out 3 families showed heterozygous pattern. One normal father also had heterozygous pattern (Fig. 107 & 108).

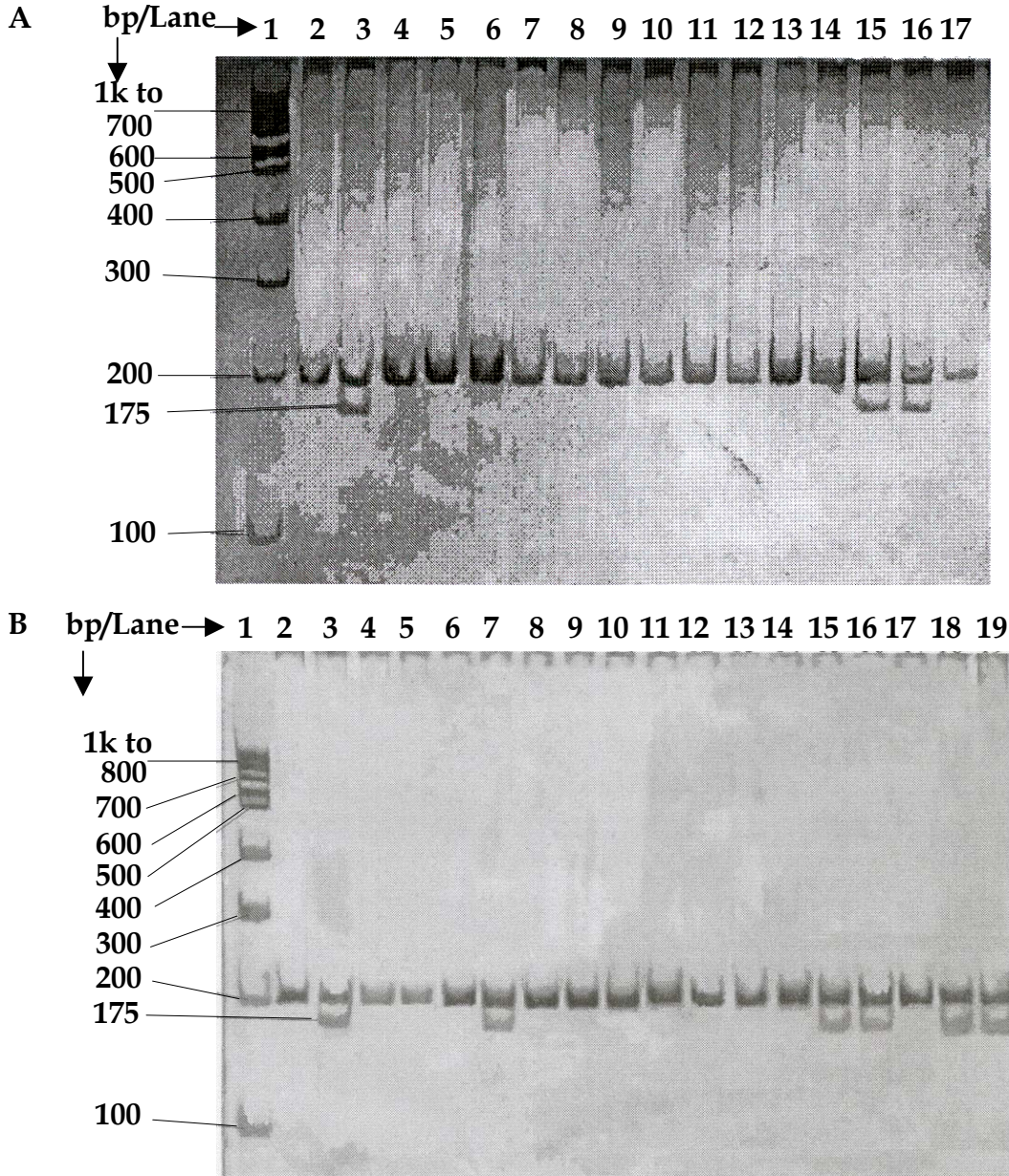


Fig. 107: (A): Lane 3: Positive control; Lane 15: Mother of Down syndrome; Lane 16: Down syndrome. (B): Lane 3: Positive control; Lane 7: Mother of NTD; Lane 15: Mother of Down syndrome; Lane 16: Down syndrome; Lane 18: Mother of Down syndrome; Lane 19: Down syndrome.

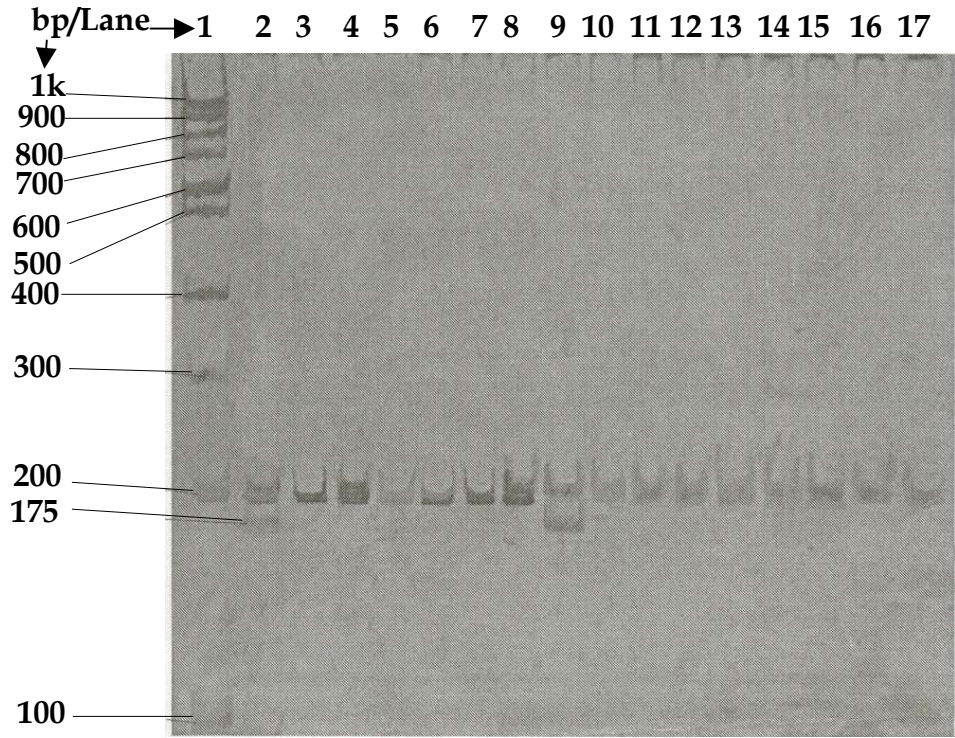


Fig. 108: Lane 3: Positive control; Lane 9: Heterozygous, other lanes 1, 2, 4-7, 10, 11-17 are normal.