GENETIC DISORDERS

Genetic disorders cause considerable burden on the family as well as the society. Therefore preventive genetics plays a crucial role in reproductive health. Our mandate is to prevent births of malformed babies by offering genetic counseling, genetic screening and prenatal diagnosis and secondly, to develop simple tests to detect carriers for thalassaemia and fragile X syndrome. 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 (FXS).

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 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

Principal Investigator: Zareen Patel
Project Associates: Shyla Menon, Rashmi Adhia, Aruna D’souza, H. Gawde, and Shiny Babu
Collaborating Centre: BMC (Bombay Municipal Corporation)
Duration: 2000-2003

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 FMR1 promoter region silences the gene.

The objectives of this study are 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; and (iii) identify new mutations.

A rapid cost effective immunocytochemical test was standardized by using commercially available antibody to FMRP. Details of protocol and analysis of 165 has been documented (Annual Report 2002-03, p 115-117)

The project was extended to include 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, Kandivali, Goregaon, Borivli, Ghatkopar, Mulund, Bhandup, Santacruz, Chembur, Vile Parle. Initially a parents' meeting was held by a Medical Social Worker along with school teachers and BMC Beat Officers were held to explain the nature of the project and method of evaluation. Pamphlets in the local languages were distributed and together with talks on the subject. Following informed consent, blood smears were collected from 344 cases of idiopathic mental retardates. In 60 individuals the antibody test was negative, suspecting Fragile X syndrome. Blood for DNA has been collected to be processed by our collaborator Dr. Thelma B.K., University of Delhi.

On clinical examination, all subjects had speech defects, delayed milestones. DNA analysis confirmed Fragile X syndrome in 3 cases out of the 52 cases screened in 3 schools. The results from the collaborator are awaited.

7.2 Cryptic Chromosomal Rearrangements amongst Couples with Recurrent Abortions in Indian Population (Funded by Indian Council of Medical Research under Functional Genomics and Molecular Medicine Program)

Principal Investigator: Zareen Patel


Duration: 2002-2004

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 recurrence spontaneous abortion (RSA) including single gene mutations, polygenic causes.

The objectives of the study are to (i) establish the frequency of cryptic chromosomal abnormalities by karyotyping followed by telomeric FISH; (ii) detect chromosomal mosaicism in blood and sperm using inter-phase fluorescent in situ hybridization (FISH).

Trypsin Giemsa banded karyotype in 118 couples (236 individuals) revealed no gross chromosomal anomalies. Telomeric FISH was standardized using To Tel Vysion-multicolour probe panel (15 combinations) and used in five normal couples and one patient. These studies are in progress to determine its role in recurrent abortions.

FISH was carried out in 28 semen samples (2,80,000 cells) from male partners of the couples with 3 or more abortions 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 28 analysed (17.86%). 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 surveys suggest a 0.3–0.5 per cent abnormal cells and so patients showing more than 0.5 per cent abnormality were considered to be contributing abnormal gametes which might be a 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. 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 (Figs. 109 A,B,C,D,E)
Fig. 109:  
A - Sperm head showing signal for chromosome 18 with nullisomy for sex chromosomes.  
B - Sperm showing signals disomic for Chromosome Y  
C - Sperms showing signals for chromosome 13 and chromosome 21 and diploid cell  
D - Sperm head showing signals disomy for chromosome 13 (2 greens)  
E - Sperm head showing signals diploid cell with two chromosome 18, chromosome X and chromosome Y