

Genetic Research Centre is the 20th permanent centre of Indian Council of Medical Research. As per the directions of ICMR, Genetic Research Centre has invested efforts towards developing an indigenous research capacity to find practical long-term sustainable solutions to the health problems the people of India face. Genetic Research Center's efforts in the last two decades have lead to the development of tools for prevention, diagnosis and management of various genetic disorders. Some of our findings are translated into policy-making action. To achieve Millennium Development Goals in no later than 2015 set by the UN, there is an urgent need to identify approaches and means to translate knowledge into effective interventions. This means better utilization of the existing tools, development of new tools for diagnosis, treatment and prevention of genetic diseases as well as working out strategies that would result in their reaching the populations in greatest need. In this context an ELISA for HbA2, which would be useful for the rural programme, for detection of B Thalassaemia has already been established by us. Keeping the above points in view over the last year, research areas included molecular basis of birth defects with emphasis on MTHFR gene polymorphisms, Chromosomal basis of reproductive loss and identifying subtle chromosomal translocations using M-FISH as our earlier studies using T-FISH did not reveal any such translocations. As a part of reproductive genetics we have evaluated sex-reversed individuals using evidence from such rare disorders dissecting important events in sex determination.

Over the past two decades Genetic Research Centre has offered prenatal diagnosis for various genetic disorders. We now aim to expand our horizon by establishing 'state of the art' facility for preimplantation genetic diagnosis. This technique avoids the need to terminate affected pregnancies, as only the normal embryos are transferred for further development in utero.

7.1 Genetic Heterogeneity of MTHFR: It's Implication as a Risk Factor for Neural Tube Defects

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Duration: 2000-2007

Neural Tube Defects (NTD) is common and severely disabling central nervous

system malformation with worldwide distribution. It is most common among Sikhs of North India and uncommon in South India. The inheritance is multi factorial with a small genetic component and a large environmental component. It arises due to incomplete closure of Neural Tube as early as 21 to 26 days during embryogenesis. Anencephaly usually results in stillborn child and babies with spina bifida lead a handicapped life depending on the size and site of the lesion. In a study conducted by MRC and ICMR, preconceptional administration of folic acid prevented such births defects in 70 percent children. However, in 30 percent children a NTD could not be prevented. Keeping in mind that folic acid is important in preventing NTD, we studied the MTHFR gene polymorphism in population in Maharashtra.

Methylene tetrahydrofolate reductase (MTHFR) is known to play a significant role in methionine metabolism. It is inherited as an autosomal recessive trait and its deficiency is seen to play a role in a number of disorders such as Bad Obstetric history, Neural Tube defects and Down Syndrome.

MTHFR catalyses the reduction of 5,10 methylenetetrahydrofolate to 5-Methylene tetrahydrofolate which is the predominant circulating form of folate and plays a role in synthesis of nucleotides, remethylation of homocysteine as well as methylation of DNA.

The gene has 11 exons on chromosome 1p36.3. Eighteen mutations have been detected so far, the most common ones being the C667T and the A1289C mutation. The MTHFR genotype is seen to interact with many drugs and increase homocystemia especially if the mother is on folate drugs. The common polymorphic allele of MTHFR occurs at the 667 nucleotide converting an alanine to a valine and causes an increase in homocysteine levels.

Maharashtra is unique because it has a heterogeneous population of different ethnic groups and socio-economic status; further there is an influx of Caucasian gene flow. In view of all the above points the objectives were to i) screen MTHFR gene polymorphism in normal population and in a cohort of patients with NTD. ii) study genetic heterogeneity with respect to C667T and A1298C mutation as a risk factor for NTD and iii) study MTHFR gene polymorphism as a risk factor for other genetic disorder.

One hundred and seventy five samples from the general population as well as certain group ethnic groups like the Parsi community as well as the Maharashtrian community has been analyzed. The homozygotes CC genotype was seen in 75 percent of the cases with a heterozygote CT percentage of 20 percent (Fig. 125).

However no homozygotes TT that indicative of a defective gene, were seen.

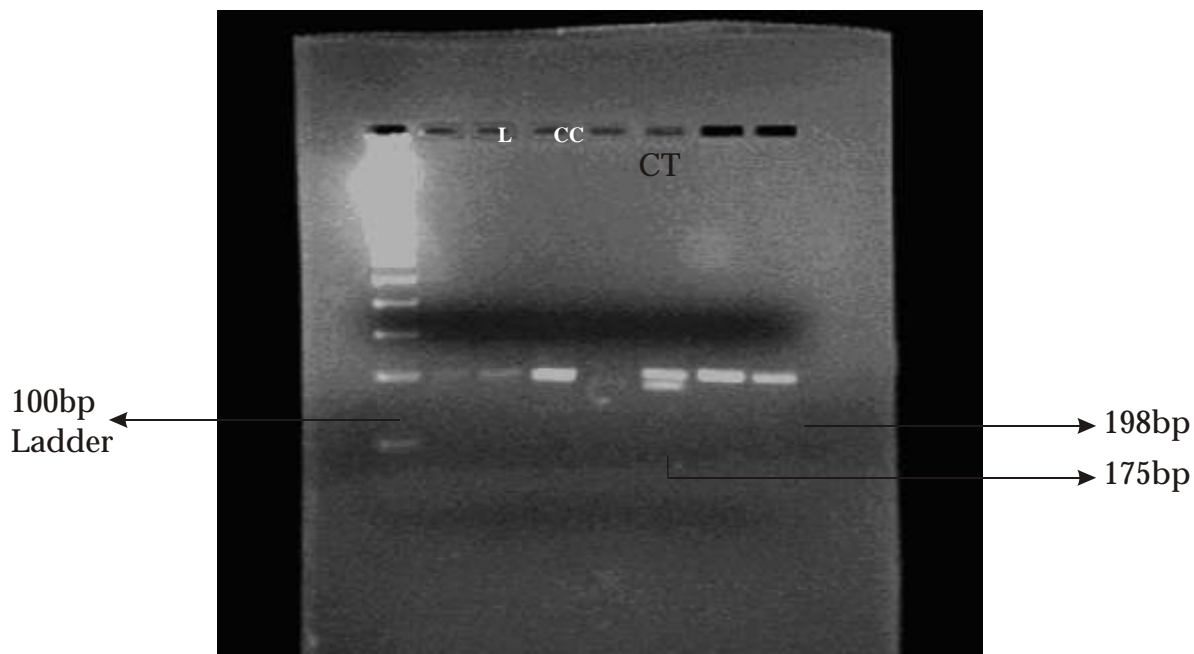


Fig. 125: PCR for C 667 T polymorphism of MTHFR Gene

7.2 Genomic Analysis of Sex Reversed Individuals

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Duration: 2005-2007

Although Y chromosome and SRY have been identified more than two decades ago the quest for sex determining genes is endless and still a debatable issue. This project was initiated with the following objectives i) is XX maleness due to X-Y interchange, if so to what extent? ii) is there a common mechanism operating in SRY Negative XX male and True Hermaphrodites? iii) are there other genes involved?

The aim was to study whether Y chromosomal DNA is present in the genome of XX males and to study genetic mechanisms involved in families with XX males and True Hermaphrodites.

Patients with ambiguous genitalia attending the clinic were evaluated clinically, biochemically and cytogenetically. Investigations included buccal smear, karyotype, ultrasonography, laproscopy, gonadal biopsy, flow cytometry, FISH and

PCR for SRY. Twenty patients were identified; the categories were classic XX males, XX male with ambiguous genitalia, XY female, True Hermaphrodites. Six cases were diagnosed as XX males, 2 with classic features and 4 with ambiguous genitalia. Two with classic phenotype were SRY positive while 4 patients with ambiguous genitalia were SRY negative. All the ten cases of XY females were SRY positive and had streak gonads. In all cases FISH for SRY was performed (Fig. 126) followed by PCR and sequencing of the PCR product. No mutations have been detected so far. Lastly, 4 cases of true hermaphrodites were diagnosed. All of them were SRY negative and had ovo testes. In conclusion, we have tried to use evidence from very rare human defects to dissect important events in sex determination. The etiology of classic XX male and XX male with ambiguous genitalia is different. Further, the etiology of XX male with ambiguous genitalia may be similar to the XX True hermaphrodites. Further studies are ongoing.

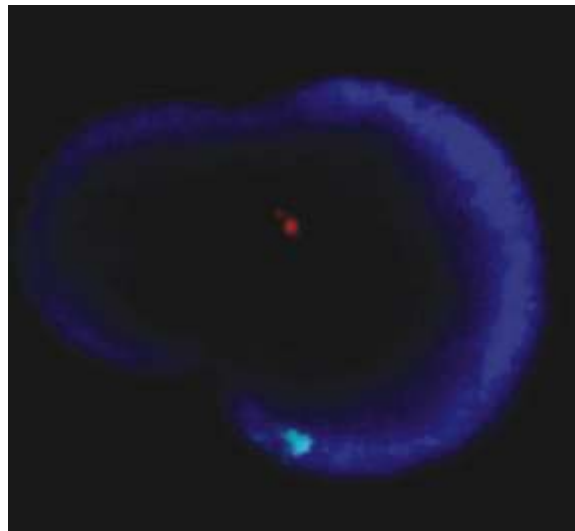


Fig. 126: FISH for SRY

7.3 Preimplantation Genetic Diagnosis (PGD)

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Duration: 2002-2007

Preimplantation Genetic Diagnosis (PGD) represents an additional prenatal service for couples at high genetic risk and has a great scope in the future. Ever since the first births were reported using preimplantation genetic diagnosis it is now performed in many countries. ART and discovery of PCR facilitated the feasibility of PGD.

Preimplantation genetic diagnosis involves testing one or two cells taken from a recent embryo of eight cells (Fig. 127a) produced by “in vitro” fertilization. This procedure is applied mainly for X-linked disease, but also for a variety of other chromosomal and single gene defects. The advantage of PGD is that it eliminates the necessity of a therapeutic abortion. The disadvantages are the requirement of “in vitro” fertilization, which has only a 15-20 percent pregnancy rate, and the experimental nature of the procedure. In fact, preimplantation genetic diagnosis requires stimulation of ovulation, which can have serious side effects. Egg collection is an invasive procedure, preimplantation is somewhat less and the success rate is still low. It is also difficult to ensure accurate diagnosis on one or two cells and the risk of misdiagnosis is higher than in other prenatal diagnostic procedures. Currently, preimplantation genetic diagnosis is offered as a very early form of prenatal diagnosis to women who are at high risk (25-50%) for having a baby with an inherited condition and who do not wish to face the possibility of pregnancy termination. It is not feasible to routinely test women at lower risks, since it would need the use of IVF for establishing a pregnancy. However, women who conceive with assisted reproductive technologies could be offered a preimplantation genetic test. For instance PGD could be used as a screening method for chromosomal disorders in all preimplantation embryos to avoid the possibility of giving birth to a child with congenital birth defects to improve the rate of pregnancies post infertility treatment.

PGD requires the expertise in the field of reproductive medicine, molecular medicine and cytogenetics. During the two decades, Genetic Centre has established all facilities for control of genetic disorders. It is proposed to expand the activity by setting up cytomolecular facilities for research in preimplantation genetic diagnosis.

Over the last year FISH studies have been initiated in blastomeres for aneuploidies and the technique standardized (Fig. 127 b). 13 blastomeres were studied.

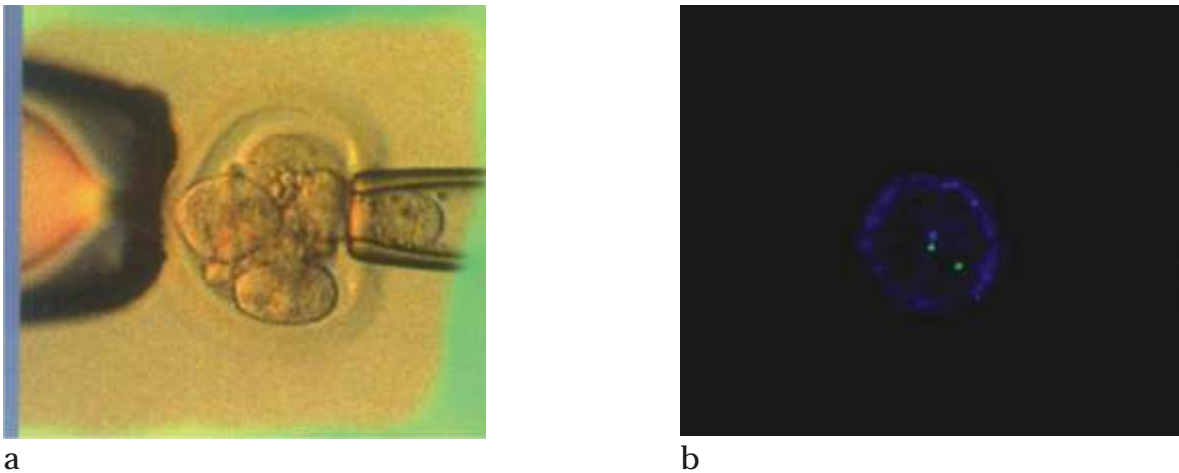


Fig. 127: a: Blastomere and b: FISH studies in Blastomere

7.4 The Role of CCR5 alleles in HIV Transmission, Progression and Prevention

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Duration: 2005-2007

AIDS is not generally considered a genetic disorder however there is heterogeneity determined by various genes, which modify virus replication and immunity. Studies in 1997 confirmed the protective role of chemokine gene receptor polymorphism in HIV transmission. A mutant allele of CCR5 prevents cell invasion by HIV. Homozygous for CCR5 mutation are highly resistant to HIV infection. Heterozygosity for CCR5 does not protect the individuals however; they have lower viral load and progress to AIDS in ten years.

A global survey indicates that CCR5 is not confined to people of European descent only but also in people through out Europe, Middle East and the Indian subcontinent with a frequency of 2 to 5 percent. The frequency of CCR5 amongst Muslim in north India is 5.3 percent. CCR5 alleles have not been studied in western India, although Maharashtra has migrants form Central Asia, Eurasia and Portugal.

HIV1 first gains entry through vaginal mucosal cell and then into the systemic circulation. A spermatozoa also carries HIV and enhances HIV transmission into genital cell. In the course of fertilization it can transmit the virus to the embryo. Hormones regulate HIV receptors in endometrium and increase the susceptibility to

infection. Normally, people have two alleles for CCR5 gene. 1.5 percent to 1.7 percent has one mutant alleles, while 1 percent has two mutant alleles. The mutant alleles have been shown to be present in 11 percent Caucasians, 17 percent Africans and 1 percent Asians. In a pilot study using established protocol and primers, 80 subjects were studied for detection of CCR5 mutation. All subjects were normal and homozygous for CCR5 as indicated by a band at 193 bp (Fig. 128).

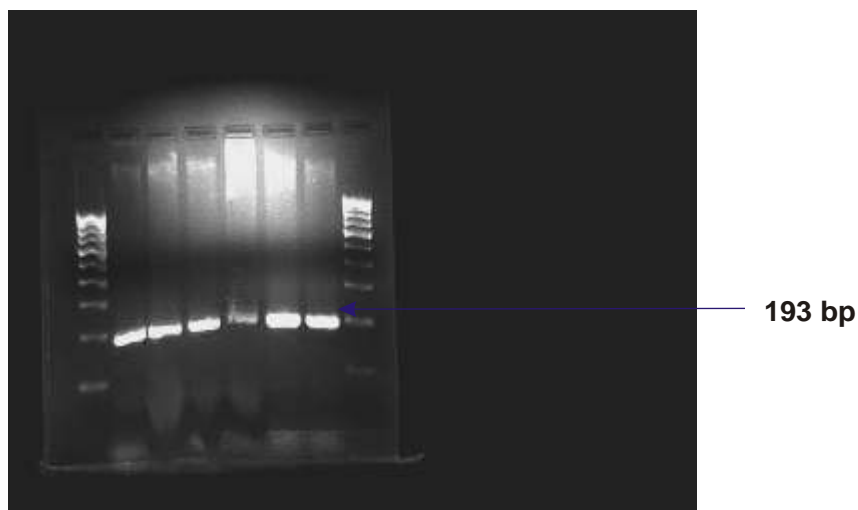


Fig. 128: PCR for CCR5 mutation

7.5 Cryptic Chromosomal Rearrangements in Couples with Three or more Recurrent Abortions

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Duration: 2002-2007

We have continued to evaluate cases of recurrent spontaneous abortions with the objectives of establishing frequency of cryptic chromosomal rearrangement by karyotyping followed by T-FISH and Sperm FISH.

Results of Sperm FISH are given in the (Annual Report 2004-05, p 162). Studies by T-FISH did not reveal any chromosomal rearrangement.

Multi coloured fluorescent in situ hybridisation (M-FISH) has a significant application whereby merely viewing the unique colour of each chromosome in a karyogram one can easily identify complex translocations, insertions and marker chromosome. M-FISH image analysis is performed by visualizing under epifluorescent microscope followed by capturing electronic images using a CCD

camera combined with digital image processing by M-FISH software. The images are visualized sequentially with single band pass filter sets. The procedure is being standardized (Fig.129).

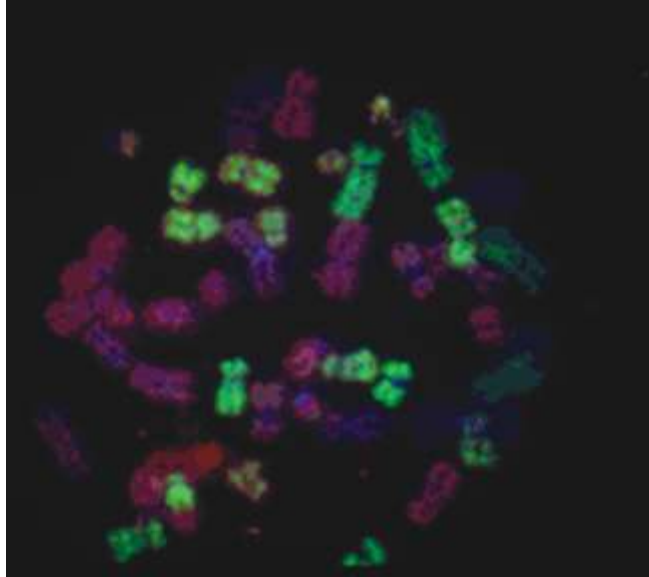


Fig. 129: M-FISH using Metaphase