

SIR GANGA RAM HOSPITAL: NEW DELHI

CENTRE OF MEDICAL GENETICS

Achievements

Research Activities

Despite being a busy clinical and laboratory service for genetic tests, research finds an important place in the departmental activities. Many projects completed in the past have added new information on genetic disorders in India. Examples include the molecular analysis of cystic megalencephaly, fragile X syndrome, genotype-phenotype correlations in thalassemia, and polymorphisms and mutations in albinism, Wilson disease, and Crigler-Najjar syndrome.

In our department the unique combination of clinical and research expertise allows the department to bring basic research from the bench to the bedside.

COMPLETED RESEARCH PROJECTS:

1. Pharmacogenomic Studies in Warfarin

A large number of people are on warfarin or coumarin derivatives for anticoagulation but it is one of the most common drugs causing adverse reactions. Determining the therapeutic dose is a trial and error, as it varies from 0.5 mg to 15 mg a day. Recent developments in predicting a patient's therapeutic warfarin dose are based on identification of polymorphisms of two genes whose products affect warfarin metabolism and warfarin inhibition of vitamin K cofactor activity – CYP2C9 polymorphic variants 2 & 3, and VKORC1 promoter – 1639 G>A polymorphism. Studies are on to investigate the frequency of these polymorphisms in the Indian population, and to investigate their effect on warfarin / acenocoumarol dose.

2. Molecular genetic studies in Male infertility

Infertility affects 1 in 7 couples. In about 50% of these male factor is responsible. It is estimated that there are 7.8 million infertile males in India. We are investigating the genetic factors leading to male infertile. Chromosomes studies, Y chromosome microdeletions, CFTR gene mutations in cases of congenital absence of vas deferens, androgen receptor gene mutations in those showing high androgen sensitivity index, and mutations studies in hypogonadotropic hypogonadism for KAL1 (Kallman gene), and FGFRI gene for cases inherited as autosomal recessive trait are in progress.

3. Molecular genetics of congenital sensorineural deafness

Genetic deafness is common and affects 1 in 1000 newborns. The commonest gene involved in causing sensorineuroal deafnesses connexin 26. We are engaged in studies in sequencing both connexin 26 as well as connexin 30 genes in cases of sensory neural deafness. We detect mutations in this gene in about 25% of cases. The attempt is to identify the mutations common in the Indian population and develop a protocol of study.

4. Pharmacogenomic studies on polymorphisms in TPMT and UGT1A1 gene in Indian population.

We intend to determine the frequency of polymorphisms of TPMT gene for use of irinotecan in colon cancer useful while prescribing 6-mercapto purine, and azathioprine, UGT1A1 gene polymorphism.

5. **HLA studies in cases of Celiac disease & their Siblings**

In this project we study the DQ2 / DQ8 haplotypes in cases of celiac disease confirmed by raised serum transglutaminase and duodenal biopsy. We also investigate siblings for any features of celiac disease, and carry out molecular tests to determine whether they have DQ2 / DQ8 allele

6. **Pattern of lysosomal storage disorders in India**

We are compiling a list of lysosomal disorder reported from different parts of India. We are also examining the distribution of cases of lysosomal disorder as diagnosed in our laboratory. This will help us to define the pattern of LSDs in India.

7. **Value of 1st Trimester and 2nd Trimester biochemical screening in India**

We have an ongoing study to determine the usefulness of 1st & 2nd trimester by biochemical screening in the Indian situation. All abnormal samples undergo amniocentesis to confirm the chromosomal constitution of the fetus. Correlation of triple test is done with ultrasound findings and results of amniocentesis.

8. **Cytogenetic studies in recurrent abortions**

To determine the frequency & balanced chromosomal abnormalities in patients who have two or more recurrent abortions.

9. **Molecular diagnosis of Megalencephalic Leukoencephalopathy with Subcortical Cysts (MLC 1)**

Van der Knaap disease or Megalencephalic Leukoencephalopathy with Subcortical Cysts (MLC 1) is an autosomal recessive genetic disorder with onset of macrocephaly before one year of age usually with progressive deterioration of motor functions. This disorder occurs due to mutations in the MLC 1 gene that codes for a putative membrane protein. We carry out studies to characterize the mutations present in the Indian patients of MLC 1. We have analyzed 27 individuals belonging to 22 families. Eighteen patients were found to be homozygous for 135insC which confirmed the diagnosis of MLC1 in these patients. They all belonged to the Agarwal community. None of the patients was found to be heterozygous. This indicates that this common mutation is due to a founder effect. This mutation was not present in four non-Agarwal families, and sequencing the gene in these cases revealed different mutations. Studies are planned to study the expression of this gene in Zebra fish and explore its functions.

10. **Genome analysis of Tyrosinase gene in Asian Indians**

Oculocutaneous albinism is an autosomal recessive disorder characterized by absence of pigment in hair, skin, and eyes. The commonest type is OCA type IA caused by mutations in the tyrosinase gene (11q14-21). We have analyzed 72 chromosomes (36 families) for mutations in OCA1A gene. The mutation R278X was observed to be the commonest Indian mutation present in 36 chromosomes (50%). Fifteen families had their child homozygous for R278X mutation and 6 families had their child heterozygous for the mutation. By sequencing of the OCA1A gene, mutations/variations were identified in 9 chromosomes. L140X a novel mutation was observed in one family. Mutations W218R and G295R observed in 2 families were found for the first time in Indian population. Intron 2+T polymorphism was observed in three cases and a SNP in exon1 was detected in one case. Rest of the 27 chromosomes are under the process of sequencing. Using this knowledge of molecular mutations we carried out 11 prenatal diagnoses in 10 families. We are also investigating mutations in OCA2 (P gene) and OCA3 gene (TYRP 1 gene).

11. **Non Invasive Prenatal diagnosis of Thalassemia**

We are already carrying out studies on fetal DNA in maternal blood to diagnose the presence of RhD gene in the fetus to help in management of patients with Rh hemolytic disease. We are now extending these studies to make a prenatal diagnosis of beta thalassemia major in couples where the paternal mutation is different from the maternal mutation. Women will be enrolled at 11 weeks of pregnancy and blood will be collected from the mother, the father and the affected child. Mutations of beta globin gene will be determined. Prenatal diagnosis will be done by chorionic villus sampling (CVS). The DNA will be extracted from maternal plasma and the paternal mutation will be tested by real time PCR. The results obtained on maternal plasma will be validated against tests on CVS.

12. **Founder Mutation in Breast Cancer gene 1 & 2**

We plan to enrol 300 women with breast cancer from our own hospital and from other hospitals in Delhi. After informed consent, their blood will be collected and DNA extracted. A family history will be taken and in those with positive family history, will be studied more intensely. In one of the affected in these families BRCA 1 & 2 gene will be sequenced. Once we find the mutations in about 25 families, we will check the DNA of all the other cases for the same mutations. This will allow us to develop a cost effective protocol to screen for mutations in BRCA 1 & 2 genes in patients of breast cancer in India.

13. **Genetic Counseling in MECP 1 gene in Rett syndrome**

Rett syndrome is a severe non-progressive neuro developmental disorder that almost exclusively affect females. The prevalence is 1 in 10000 female births. We have started to study the mutations in the MECP 2 gene in females with classic features of Rett syndrome. The patients are examined and Rett syndrome diagnostic criteria are checked against those prescribed by Rett Association. All the exons of gene are sequenced. In cases where we do not find a mutation MLPA, studies are done to check the presence of deletion. We have studied 15 families so far. It is proposed to study 100 families to obtain the pattern of mutations and deletions in India to help in genetic counseling of these patients.

List of Current Ongoing Research Projects

1. Molecular characterisation of organic acidurias using targeted next generation sequencing.
2. Establishing Center For Education And Training In Genetic Medicine
3. A Web Based Directory Of Genetics Services In India (Geneticsindia.Com)
4. Establishing Center for Education and Training in Genetic Medicine
5. Molecular Characterisation Of Familial Hypercholesteremia In Indian Population
6. Genetic Studies In Indian Patients With Autosomal Dominant Polycystic Kidney Disease
7. Non Invasive Prenatal Diagnosis Of B- Thalassemia By Digital Pcr Using Circulating Cell Free Fetal DNA
8. Molecular Genetic Studies In Families With Life Threatening Cardiac Arrhythmias
9. Array Genomic Hybridisation In Prenatal Genetic Diagnosis In The Indian Setting
10. Pre implantation genetic diagnosis for chromosomal disorders.

Future Plans:

We plan to setup *Pre-implantation genetic diagnosis* due to the presence of a strong IVF center in the hospital. We also plan to increase our diagnostic abilities with the installation of *the 8 capillary Sequencer*. Establishment of *Stem cell and Myoblast transfer technology for therapy of muscular dystrophies* is underway.