# 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.


**3 years DNB Super Specialty Medical Genetics Program**

Sir Ganga Ram Hospital initiated a 3 years DNB Super Specialty program in association with National Board of Examination (NBE) and Department of Biotechnology (DBT), Government of India. As a part of this program the first ever DNB in Medical Genetics is now available in the country. Students with MD (Paediatrics), MD (Medicine) & MD/MS (Obstetrician/ Gynecology) are eligible for this program. It trains students in diagnosis and management of patients and families with inherited disorders as well as all aspects of fetal medicine including prevention of genetic disorders.

**List of Current ongoing projects**

| Sr. No. | Name of Project |
|---|---|
| 1 | Characterization of Mutations in Bruton Agamma-globulinemia: A pilot study |
| 2. | A Study of Whole Exome Sequencing in Anomalous Euploid Fetuses |
| 3. | Molecular Studies of Merosin Deficient Congenital Muscular dystrophy in Indian Patients and their Clinical Application-A Preliminary Study |
| 4. | A Study of Genetics Factors in Early Fetal Growth Restriction (FGR) |
| 5. | Whole Exome Sequencing in Perinatal Lethal Skeletal Dysplasia |
| 6. | A Study of Immune Cells and Cytokines in Recurrent Pregnancy loss – A pilot study |

III. Fellowship Program – DNB in Medical Genetics / PhD (list with year/thesis title/name of the fellow and Supervisor)

| Sr. No. | Fellowship Program (Fellowship in Medical Genetics/ DNB/PhD) | Joining Year | Thesis Title | Supervisor |
|---|---|---|---|---|
| 1 | **DNB** | Apr-15 | Clinical and Genetic Studies in Pompe Disease | Dr I.C. Verma |
| 2 | **DNB** | Apr-15 | The outcome of fetuses with increased nuchal translucency in the first trimester | Dr Ratna Dua Puri |
| 3 | **DNB** | 2016 | Immune cells and cytokines in recurrent pregnancy loss-A pilot study | Dr I.C. Verma |
| 4 | **DNB** | 2016 | A Study to Estimate the Carrier Frequency of Common Genetic Disorders in North India | Dr. Sunita Bijarnia Mahay |
| 5 | **DNB** | May-17 | A study of genetic factors in early fetal growth restriction | Dr. Sunita Bijarnia Mahay |
| 6 | **DNB** | May-17 | A study of whole exome sequencing in anomalous euploid fetuses | Dr Ratna Dua Puri |
| 7 | **PhD** | 2011 (Completed last year) | Molecular Characterization of Organic acidurias in Indian patients and their clinical applications | Dr I.C. Verma |
| 8 | **PhD** | Oct'11 (Ongoing) | Molecular Characterization of Familial Hypercholesterolemia in Indian Population | Dr. Renu Saxena |
| 9 | **PhD** | Oct'11 (Ongoing) | Genetic Studies in Indian patients with Autosomal Dominant Polycystic Kidney disease | Dr. Jyotsna Verma |
| 10 | **PhD** | Oct'11 (Ongoing) | Exploration of methods for noninvasive prenatal diagnosis of beta thalassemia from circulating cell fetal DNA in maternal plasma. | Dr. Renu Saxena |
| 11 | **PhD** | 2012 (Ongoing) | Molecular Genetic Studies in Families with Life Threatening Cardiac Arrhythmias | Dr. Ratna Dua Puri |
| 12 | **PhD** | 2013 (Ongoing) | A Study of Combined Classical Cytogenetic and Microarray in Dysmorphosm, Developmental Delay, Autism, Mental Retardation and Multiple Congenital Anomalies | Dr. Meena Lall |

**SOME OF THE RESEARCH PROJECTS OF INSTITUTE OF MEDICAL GENETICS & GENOMICS ARE LISTED BELOW**

1. **<u>Pharmacogenomic Studies in Warfarin</u>**
   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 sensoreneuroal 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 $1^{st}$ & $2^{nd}$ 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.

14. **Molecular Characterization of Familial Hypercholesterolemia in Indian Population**

Familial hypercholesterolemia (FH) is the most common disorder of inherited lipid metabolism. It accelerates the onset of atherosclerotic cardiovascular disease (CVD), especially coronary heart disease (CHD), by one to four decades. Early detection and effective treatment of FH can prevent CHD in the affected individuals and result in an improved clinical outcome. FH is primarily caused by mutations in the low-density lipoprotein receptor (*LDLR*) gene that clears LDL particles from plasma, apolipoprotein B-100 (*APOB*), which is the only ligand carrying LDL cholesterol (LDL-C) from circulation to hepatocytes and proprotein convertase subtilisin / kexin Type 9 (*PCSK9*) gene, which degrades mature LDL receptors on cellular membrane. FH exists in two forms: Homozygous, the more severe form with age of onset in childhood and Heterozygous, less severe and age of onset after third decade of life.Detection based on mutation analysis can establish definitive diagnosis of FH and enables **Cascade Screening** by a systematic family tracing, which is very cost effective. Individuals can be diagnosed at an early stage of FH or even when they are asymptomatic, thereby allowing early intervention including lifestyle modification, cholesterol-lowering medications, and management of other major cardiovascular risk factors. The study funded by Research development program, Sir Ganga Ram Hospital (Grant No: EC/10/10/197; 4.9.13) and Indian Council of Medical Research (Sanction No: 54/16/2011-BMS) and Next Generation Sequencing (NGS), partially funded by Medgenome Labs Pvt. Ltd. was conducted from 2011-2015. The study enrolled 100 clinically diagnosed cases of FH along with 216 family members, classified them according to Dutch Lipid Clinic Network (DLCN) criteria and identified causative mutations, by analyzing the three candidate genes. Pathogenic mutation was identified by sanger sequencing and NGS in 47 cases. Cascade screening led to identification of 88 new cases, with a pathogenic mutation, who were at a very high risk of developing premature CAD. To our knowledge, this is the first study of its kind from India to classify subjects according to DLCN criteria, carry out mutation analysis in candidate genes and performing cascade screening in families of mutation positive probands.

15. **Molecular Genetic Studies in Families with Life Threatening Cardiac Arrythmias**

Life threatening cardiac arrhythmias are a leading cause of sudden cardiac death (SCD) in individuals with a structurally normal heart. The prevalence of these life threatening arrhythmias has been estimated to be about 1 in 10,000 to 15,000 in India. The two common arrhythmias are Long QT syndromes and Brugada syndromes.

Commonly, patients with these disorders present with aborted cardiac arrest, or with syncope, palpitations or seizures. Genetic testing confirms the clinical diagnosis and provides knowledge crucial for patient's effective treatment and lifestyle modifications. Treatment as mentioned above can be tailored depending on the location and type of mutation in the particular gene associated with specific syndrome. Identification of the mutation in the family can help in cascade screening of family members who are symptomatic as well as asymptomatic. About 10-37% of LQTS mutation carriers are asymptomatic, even have normal QTc values (30-40%) in ECG at rest, but at-risk of SCD, which can be prevented by molecular tests and timely treatment. If a parent is clinically diagnosed with LQT syndrome, then molecular testing can help in pre-natal diagnosis. Early diagnosis can help in preventing SIDS caused by LQTS syndrome by neo-natal ECG screening program as well as taking necessary precautions after birth. The identification of the mutation-positive individuals will allow them to take necessary actions and precautions and also help mutation-negative individuals to learn that they are not at-risk and will relieve them from being worried. This study is the first cohort study in an Indian population and the results are an important genetic reference for LQTS and BrS in India. It expands the spectrum of mutations and also reports on the first molecular study on BrS patients of Indian origin. Common mutations as hotspots were identified which can help in faster genetic testing. Majority of mutations identified in this study were novel. Hence, functional studies are warranted to confirm tha pathogenicity of these variations.

**16. Multi Centric Collaborative Study of the Clinical, Biochemical and Molecular Characterization of Lysosomal Storage Disorders**

LSDs are a heterogenous group of rare disorders characterized by accumulation of waste products in the lysosomes which result in cellular dysfunction and clinical abnormalities. This occurs due to absence or deficiency of lysosomal enzymes which help in breakdown of macromolecules. We have an ongoing multicentric study on Lysosomal Storage Disorders (LSD) funded by Department of Health Research (DHR). The primary objectives of the study are:

1. To identify pathogenic mutations in LSDs and study genotype phenotype correlation and do molecular modelling for novel variants
2. To establish the utility of newer biomarkers for diagnosis and prognosis of LSDs
3. To establish a database of the mutations and sequence variations found in Indian patients with LSDs.
4. To standardize the diagnosis of lysosomal storage disorders using dried blood spots

There are many lysosomal storage disorders. At our centre, we are studying Pompe Disease, Multiple sulfatase deficiency and Aspartylglycosaminuria under the DHR funded project.