# REPORT

1. Name and designation of ICMR-IF : Dr. Babu Rao Vundinti, Scientist-F

2. Address : ICMR-National Institute of Immunohaematology
   13th floor, New multistoried building, K.E.M
   Hospital campus, Parel, Mumbai-400012, MS,
   India.

3. Frontline area of research in which Training/research was carried out : Molecular Genetics of Fanconi Anemia

4. Name & address of Professor and Host Institute : Dr.Settara Chandrasekharappa, PhD
   Head, Cancer Genomics Unit, CGCGB,
   Director, Genomics Core
   National Institutes of Health
   National Human Genome Research Institute
   50 South Drive, Building 50, Room 5232
   Bethesda, Maryland 20892, USA

5. Duration of fellowship : 15 days

6. Highlights of work conducted :

   i) **Techniques/expertise acquired:** Understanding the molecular mechanism of Fanconi anemia (FA) is one of my current research area. FA is a rare genetic disease and associated with 22 genes. Though clinical phenotype presented with several clinical abnormalities, major clinical abnormalities are skeletal anomalies, skin pigmentation and Bone marrow failure. The FA cells are sensitive to alkylating agents. The chromosomal breakage is gold standard for the diagnosis. The FA genes (proteins) function in FA pathway. Defect or mutation in any of the genes leading to genomic instability as these proteins fails to repair the DNA damage. The FA patients high risk to developing cancers. Our laboratory only the center to carry out research on FA in India. We have developed recent molecular procedures to understand FA pathway. In our experience at least 20% of FA subjects presented with somatic mosaicism by chromosomal breakage. The review of literature suggests that the somatic mosaicism due to gene reversion or also called natural gene therapy. The molecular evaluation of somatic mosaicism is important to treat the FA subjects.

   The aim of the training was to understand the molecular mechanism of somatic mosaicism in Fanconi Anemia (FA). Establishing the somatic mosaicism by chromosomal breakage is important for further molecular characterization.

Though I have expertise in this field, I have taken FANCB cell line at host Institute and studied for mosaicism. In this cell line atleast 25% cells showed no chromosomal breakage and it indicates that the FA subject progressed to somatic mosaicism as at the time of diagnosis the FA subject presented with 100% chromosomal breakage in cells.

The training majorly involved with Next Generation Sequencing (NGS) technology to study mutation regions and understanding reversion of gene. During the fellowship period, I have been trained in designing the probes, capture, enrichment, library preparation and sequencing. A total 5136 100mer molecular inversion probes (MIP) designed to capture the entire genomic region plus 1-kb flanking regions for the FA and related genes. Sequencing of the enriched libraries was performed on an Illumina GA-II platform in a single end 36 base configuration.

The knowledge gained in designing the probes for various regions of FA genes, which are used for NGS. The other important advanced technology is array-comparative genomic hybridization (aCGH). During the fellowship I have exposed to aCGH technology to identify copy number changes. Briefly Agilent CGH array was designed to query the entire length and extended regions of all FA and other inherited bone marrow failure syndrome genes. The experimental procedures are briefly, genomic DNA from the patient and a reference male DNA were differentially labeled and hybridized to the array. The analysis of NGS data is important part in identifying genomic changes. I had spent some time with Bio-informatics to understand the analysis of NGS data. The alignments stored in BAM format were used for genotype determinations, including single-nucleotide and deletion/insertion variants, using the most probable genotype algorithm. Genotypes were considered high quality if the most probable genotype score was $\geq$10 and the score divided by the coverage was $\geq$0.5. VarSifter , a versatile software that can display and allow for sifting through sequence variants by both inclusive and exclusive criteria, was used for evaluation of sequence data. We chose to view unique exonic deleterious (nonsynonymous, indel, splice) variants by excluding those present in dbSNP in all the FA genes. If we did not find 2 variants within a sample in an FA gene, the search was then extended to include synonymous changes and variants in the introns while excluding those present in dbSNP. The technology used for the study of FANCB cell line which is established as reversion of mutation. I have also used my time to see the zebra fish core laboratory. The culturing of fish and FA models of zebra fish was observed. The technology used to develop animal model of FA was very interesting, hopefully this knowledge will be used for our future studies.

ii) Research results, including any papers Prepared/submitted for publication: Nil

iii) Proposed utilization of the experience in India : We have ongoing projects on molecular characterization of Fanconi anemia. The training in the area of NGS especially analysis of NGS data is very useful for identifying and characterization of novel genes. The training is enormously helpful in understanding the somatic mosaicism in Fanconi anemia. The technology we will apply to our FA subjects to see the natural correction or reversion of mutated gene in our FA cohort. This will help in proper management of the disease. Exposure in Zebra Fish core lab will help largely in designing future projects to understand molecular mechanisms in various disease conditions.

ICMR Sanction No. INDO/FRC/452/(S-20)/2017-18 IHD

Signature of ICMR-IF