Centre for Brain Research

An autonomous Centre of the Indian Institute of Science

ANNUAL REPORT
2017-18

Mandate and Vision

I. To Carry out basic research to understand brain function in health and disease.

II. Foster focused research programs for inter-disciplinary neuroscience research to discover rational therapies for dementia and other age-related brain disorders.

III. Networking of neuroscientists among institutions across the country, with emphasis on neurodegenerative disorders.

CBR is an autonomous centre of the Indian Institute of Science funded by Pratiksha Trust (set up by Mr. Kris Gopalakrishnan, co-founder of Infosys and Mrs. Sudha Gopalakrishnan). CBR activities are currently carried out in a temporary location within IISc. The centre will move to its own building on 50,000 sq.ft of land identified for this purpose within the Institute campus. The planning for the building has been finalized with architects and the construction work should commence soon.

Research activities at CBR

I. SANSCOG Project

Project head coordinators: Prof. Vijayalakshmi Ravindranath (CBR), Prof. BN Gangadhar (NIMHANS).

Investigators: Prof. PT Sivakumar (NIMHANS), Dr. Naren Rao (NIMHANS), Dr. Ganesh Chauhan (CBR), Dr. Bratati Kahali (CBR), Dr. Smitha Karunakaran (CBR), Prof. G Rangarajan (IISc), Prof. Y Narahari (IISc) among others.

CBR, in collaboration with National Institute of Mental Health and Neurosciences (NIMHANS), Sri Devaraj Urs Medical College, Kolar and the Indian Institute of Science (IISc) has started the Srinivaspura Aging Neuro Senescence and COGnition (SANSCOG) study. The SANSCOG study is envisioned as a prospective community based cohort study with long term follow-up over many years for comprehensive evaluation of risk and protective factors associated with cognitive changes due to normal ageing, Alzheimer's disease and other related disorders. This will be a cohort of 10,000 individuals from the Srinivaspura taluk of Kolar district in the state of Karnataka aged over 45 years and the pilot study which is currently under way aims at recruiting 1,000 individuals.

Awareness camps followed by home visits:

Prior to beginning of recruitment, the people of the area need to be sensitized about mental health and the need of the current study. We have chosen Dalasanuru, one of the public health center (PHC) in Srinivaspura as our initial recruitment area. So far 11 awareness sessions have been conducted in this PHC and close to 675 people above the age of 45 years have attended these sessions. Audio-video clips and photographs with simple messages are used to convey the issues related to mental health and how this study would address some of the aspects my detailed medical examination of the people of Srinivaspura taluk. During the awareness talks people were also asked if they were interested in participating in the study and their contact details were noted. This was followed by visiting the house of the subjects who had shown interest in the study. During this home visit if the subjects agreed, then their written informed consent was taken with a detailed description of the various tests that they will have to undergo. They were also told that they will have to come for annual follow-up. During the home visits along with the consent form, their personal contact information was also collected along with filling of a short socio-demographic questionnaire. The home visits started in the first week of January 2018 and so far, approximately 200 home visits have been completed. Majority of the subjects who were willing to participate in the study during the awareness camps have given their written consent and participated in the study when they were visited at their homes. Currently we have >80% conversion rate from willingness to consent, which is quite high compared to what has been observed in other studies.

Detailed medical examination at SANSCOG health centre at Srinivaspura:

The home visits are followed by a visit to the health centre office of the SANSCOG project located in Srinivaspura town. During these visits the subjects undergo a detailed medical examination which includes anthropometry and gait measurements, ECG, blood pressure measurements, eye fundus photographs, etc. This is followed by a detailed neuropsychiatric examination to detect various neurodegenerative diseases along with family history and medication records. Lastly, they undergo a detailed cognitive examination that covers various domains of the brain function. A team of medical doctors including psychiatrists, nurses, psychologist, social workers and other support staff manage this SANSCOG office. The transport of the subjects to the centre and the breakfast and lunch are taken care by CBR. So far over approximately 150 subjects have completed the detailed medical examinations at the centre.

Blood test camps:

We conduct camps in the villages from where the subjects are recruited to collect their blood samples for a detailed blood biochemistry examination and one aliquot is used for isolating DNA for genetic studies. Routinely the blood camps

are organized every two weeks where close to 50 subjects who have finished their detailed medical examination are invited. Periodic organization of these camps helps us avoid inter-day variations and managing of other logistics. After the camps the subjects are given breakfast, an honorarium and a bag for participating in the study. So far 103 people have attended these camps and given blood.

Blood collection: The blood collection is managed by the SANSCOG team. The phlebotomy team was provided with the names, bar codes, phone number, age and gender of consenting respondents. Collection materials are taken to the site of blood draw camp at respective villages. Every attempt is made to schedule blood draw twice a month preferably during the second and fourth week. Fasting was recommended. The blood tests are done at Sri Devaraj Urs medical college in Kolar.

No	Date	No. of subjects enrolled for each camp	No. subjects arrived for blood test	Location of camp	Name of the village
1	26-03-18	104	62	Primary Health Centre	Dalasanuru
2	28-03-18	46	41	Primary Health Centre	Dalasanuru
		Total	**103**		

Assays: The list of blood parameters that were monitored is presented in the table below:

Sl.No.	Tests
1	Cell counts, (total leucocyte count, platelet count & haemoglobin)
2	Fasting Blood Glucose
3	HbA1C
4	HDL-Cholesterol
5	LDL-Cholesterol
6	Total Cholesterol

7	Triglycerides
8	Blood Urea
9	Blood Creatinine
10	Bilirubin
11	Alanine transaminase (ALT)
12	Aspartate aminotransferase (AST)
13	Alkaline phosphatase (ALP)
14	C-Reactive Protein (CRP)
15	Vitamin B12
16	Folate
17	Homocysteine
18	Vitamin D
19	Insulin
20	C-peptide
21	T3, T4, TSH

Results of the assays were provided to the participants within 1-2 weeks of blood collection. Further, the results were monitored for alert values and the physicians at Srinivaspura site were notified of any severely out of expected range results.

Quality Control: For internal quality control purpose, and to further monitor the inter and intraday variation with analysis and reliability of basic biochemical parameters at the clinical biochemistry lab at SDUMC, we performed duplicate analysis with healthy volunteer blood samples (n=4). The samples were barcoded like regular test samples. The results obtained were assessed for the similarity of the values and their distributions. In general, the inter and intraday results were quite similar for majority of the assays.

The central diagnostic laboratory at SDUMC is NABL (National Accreditation Board for Testing and Calibration Laboratories) accredited and has standard quality control procedures to ensure high quality of performance of laboratory procedures. Samples are stored in freezer provided by SANSCOG project to SDUMC. The personnel appointed by SANSCOG monitors the assays and maintains the records. Internal quality control at SDUMC is maintained by routine calibration of the instruments with standards, analysis of control sample with each analytical run and monitoring of control pool values.

Digitization of questionnaire and the entire work-flow:

The entire work-flow of the project has been digitized so that all medical examination and questionnaires that are part of the study are administered through a tab or via a web browser on a laptop or computer. We have used open source tools to create the platform with the central database being located at CBR, IISc.

The open source tools used for building this platform are presented below. It is a three-stage design system:

Stage-1, Data collection: The "ODK Aggregate" suite serves as the platform for data collections form various sources like the FDC in the field, nurses, doctors and various other sources.

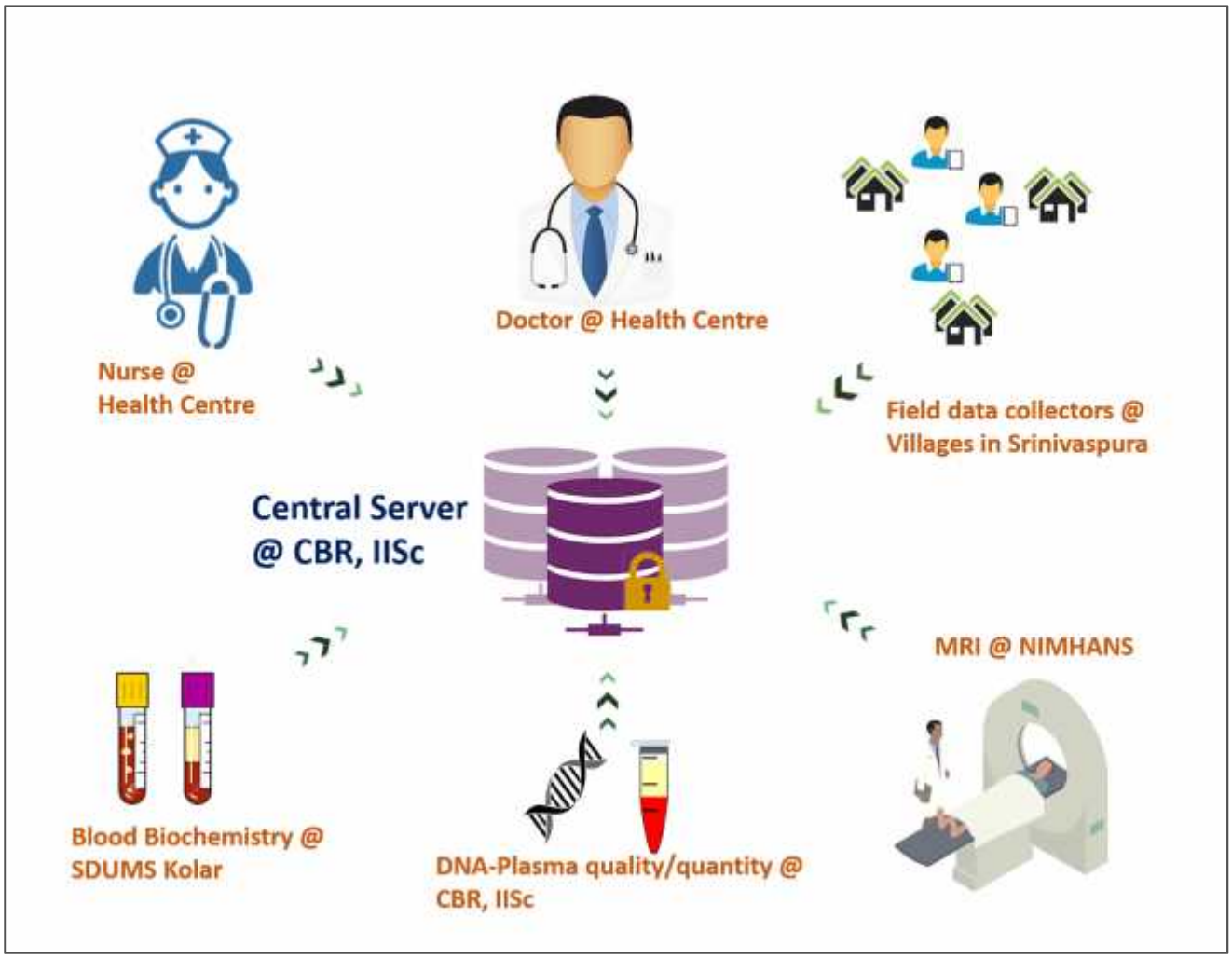
Stage-2, Data QC: Selective data is then passed on to "ODK 2 Tables" and selective people are given access to the data to view and correct mistakes.

Stage-3, Data repository: The "OpenClinica" platform is used for holding the final data set after qc. It is also the source for sharing data with various investigators for various analysis.

Open source platforms used to create the platform for SANSCOG data management

The various elements of digitization include capturing these various processes:

Centralized data capture using the platform:



Visits to the site by international experts:

To get critical review of the study so far, we have invited two senior researchers who are experts in the field of dementia and have run large cohorts of dementia and related disorders. Our first expert was Prof. Mary Ganguly from the University of Pittsburg who is a professor of psychiatry, neurology and epidemiology and was among the first researchers to start a population based study of dementia in India. Our second expert was Prof. Howard Feldman who is a Neurologist and Professor of Neurosciences at the University of California, San Diego and Director of the Alzheimer Disease Cooperative Study and has led many drug trials including those for Alzheimer's disease. These experts were with us for 5 days each to review the study protocols in detail. These experts visited the Dalasanuru PHC and participated in the awareness sessions and visited the field office located in Srinivaspura where all detailed medical examinations will be held. They have given important suggestions to improve the study without diluting the scientific content and the logistic challenges associated with large scale prospective population based studies. All the advice given by these experts are currently being adopted and the protocols have been modified accordingly. Experts pertaining to other domains of the study like MRI and genetics will visit us in the coming months.

Visit by Mr. Kris Gopalakrishnan & others and signing of MoU with SDUMC:

On the 19th of Feb 2018, Mr. Kris Gopalakrishnan along with his wife Sudha Gopalakrishnan, Prof. B.N Gangadhar (Director, NIMHANS), Prof. Vijayalakshmi Ravindranath (Convener, CBR) and Dr. Saraswati Padmanabhan (Representative, TATA Trusts) visited the SANSCOG study site in Srinivaspura. They attended an awareness talk in one of the village from where we recruit subjects. Later they moved to the SANSCOG office in Srinivaspura where we conduct the detailed medical examination. There we were joined by the Chancellor and Registrar of Sri Devaraj Urs Medical College (SDUMC), Kolar. A tri-patriated agreement was signed between CBR, NIMHANS and SDUMC. TATA Trust have also agreed to provide medical care for the elderly population who are part of the SANSCOG project including the Digital Nerve Network (DiNC) program that they are running in the Kolar district.

The successful execution of this pilot phase will pave the path for implementation of the SANSCOG study over 10 years with the aim to identify reliable markers to predict the risk for development of dementia. This will also create a platform of multi-disciplinary approach to study brain aging in the Indian population.

Association with the TATA Trusts:

1. Convergence of research activities of CBR with the current Tata Trusts project at IISc.

The idea is that the research activities can proceed seamlessly and enlarging of the research goals can be thought off.

For example, there is a small urban cohort (Bangalore cohort) where information is collected on about 300 human subjects under the Tata project. This is similar to the work being done at Kolar under CBR, where it is proposed to follow up 10,000 subjects. The urban cohorts could be expanded so that the rural and urban cohorts can be compared in terms of risk and protective factors.

The activities under the computational analysis can be enlarged with the help of IISc faculty and develop methods for looking at the data from both Kolar and Bangalore cohorts.

Tata Trusts discussed about the renewal of the Tata grant in 2019 and wanted to know if the proposal for renewal could be made jointly so that both IISc and CBR faculty could participate in the grant.

2. In terms of CBR working with the Tata Trusts, two ways were thought off.

a) Tata trusts could provide medical services for the Kolar cohort that CBR is studying. There were discussions about setting up a mobile van, wherein the tests could be done in the villages itself rather than the subjects coming to CBR site office. Tata trusts have offered to integrate the medical services in the mobile van.

b) Tata trusts are providing health care for elderly in 3 districts in the country in collaboration with the government. It was felt that the questionnaires developed by CBR (administered through tablets) could be used. In short, the tools developed by CBR could be shared and CBR could also potentially recruit the elderly for research studies.

c) Tata Trusts are also working with Karnataka Government to provide medical services and keep the records through a large digitization initiative. This is being done in Kolar district. They have agreed to prioritize this process in those villages that CBR has initiated research work.

d) The Genome India initiative started by CBR has now participation from 14 institutes across the country. The PSA's office has also indicated seed funding. Considering the interest of Tata Trusts in cancer genomics, potential partnership with the Trusts for Genome India also needs to be initiated. A concept note on this matter has been sent.

II. GenomeIndia

Investigators: Dr. Bratati Kahali (CBR), Dr. Ganesh Chauhan (CBR), Prof. Vijayalakshmi Ravindranath among others.

CBR is currently spearheading the GenomeIndia initiative- a first of its kind in the country aimed at cataloguing the genetic variation in Indian individuals by information from deep whole genome sequencing (WGS) of 20,000 individuals represented by diverse ethnic and linguistic groups across various geographical regions of the country, with collaboration from several national institutes across India (A pictorial representation of the partners is given in Figure below). Since genetic variations play a major role in health and disease, a systematic study of the genetic heterogeneity of the Indian population and numerous sub-populations is crucial to identify the complex and rare disease risk factors in Indian population. Complete information over the genome, including haplotype information, common, low frequency and rare genetic variants, and copy number variations for Indian populations will be catalogued. The availability of a comprehensive catalogue of genetic variations from India will serve the immediate purposes of (i) developing a genome wide association chip to do genetic studies at whole genome level at a cheaper cost in India (ii) develop a reference panel for imputation for comprehensive genetic studies and (iii) help perform genetic studies on monogenic disorders as the comprehensive list of genetic variation obtained from healthy individuals will act as filter for non-causal mutations. CBR will sequence the whole genomes of 1000 individuals with the aim of mapping the haplotype structure and genetic variation as part of this pan-Indian project. Besides, CBR has also designed the standard operating procedures for sample collection with background information after an individual has consented to participate in the study. CBR is also leading the analyses of whole genome sequence data for the entire project.

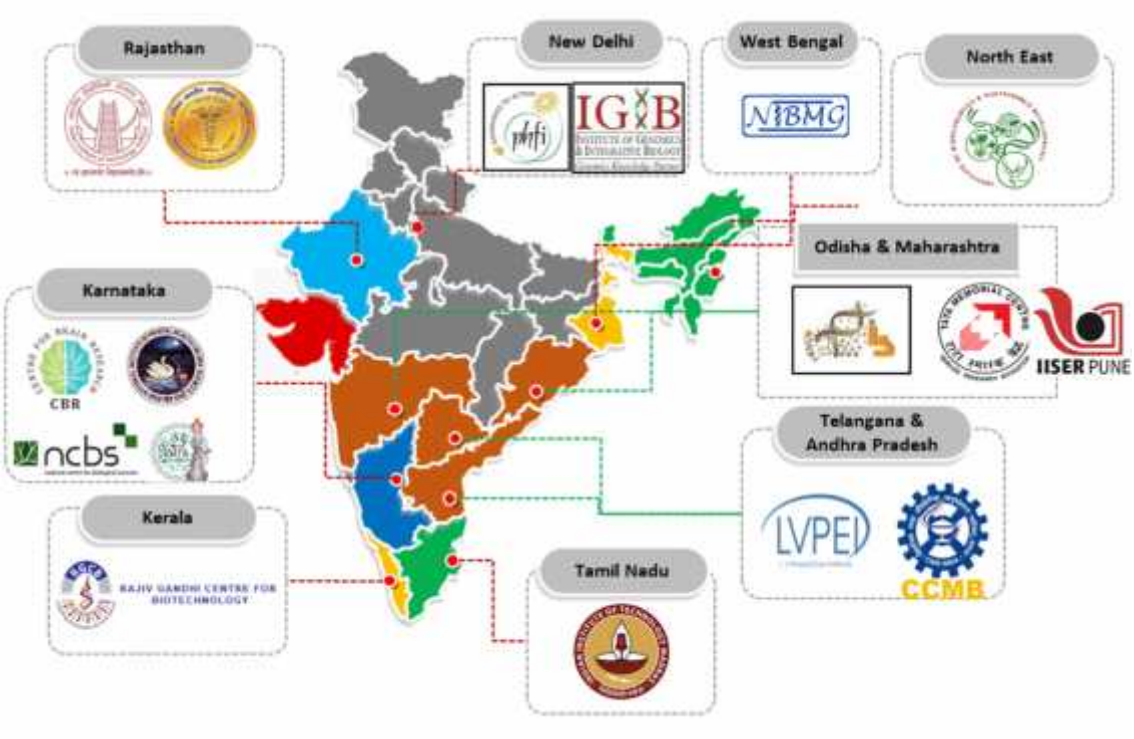


Figure. Collaborative partners of GenomeIndia across the country

Some recent updates for this project are:

a. The standard operating procedures for sample collection with background information after an individual has consented to participate in the study has been clearly laid out. These include collection of brief demographic and epidemiological details of the participants, including age, gender, education, marital status, occupation, past history of illness/infections, medication use checklist, and exposure to hazardous chemicals. Detailed anthropometric and physical examination (including height, weight, waist circumference, hip circumference, head circumference and blood pressure) are also being collected.

b. The standard operating procedures for collection of 10ml blood for genetic studies and biochemical investigations from each study subject have also been adopted by collaborating institutions.

c. Most of the collaborating institutions have obtained ethical clearance.

d. Some individuals from urban Bangalore have consented to the study and their DNA have been isolated from whole blood for WGS.

e. CBR have optimized the procedure for data analyses from WGS of individuals by analysing publicly available dataset of raw data from WGS at our workstations.

f. Four working group meetings have been held as of March 2018. The venues were IISc- Bangalore, LVPEI- Hyderabad, IBSD-Imphal, NIBMG- Kalyani. The meeting at IBSD also had two workshops focusing on DNA isolation best practices, and computational analyses of whole genome sequencing data in order to educate the local community of scientist, researchers and medical professionals in the North-East.

g. Prof. Ravindranath and Dr. Chauhan have visited several whole genome sequencing centers in the US prior to engaging in sequencing for GenomeIndia. We have strategic partners at BROAD Institute, University of Pennsylvania, Craig Venter Institute at the US.

h. A Next Generation Sequencing platform is being set up which is funded by the TATA Trusts.

III. "Alzheimer's disease: Understanding Mechanisms for Early Diagnosis and Treatment"

Faculty of CBR have also joined the ongoing research project funded by Tata Trusts on "Alzheimer's disease: Understanding Mechanisms for Early Diagnosis and Treatment" at the Centre for Neuroscience, IISc. The individual research focus of CBR faculties Dr. Ganesh Chauhan and Dr. Bratati Kahali under this project are provided below.

IV. The individual research focus of CBR faculties

1. Dr. Bratati Kahali

Project title: Decoding genetic basis of metabolic disorders and its shared etiology with dementia in Indian population

This project is funded by the Ramalingaswami Fellowship awarded to Dr. Kahali.

Investigator: Dr. Bratati Kahali

Polygenic and complex human disease like, dyslipidemia, type 2 diabetes (T2D), cardiovascular disease, neurodegenerative disorders, fatty liver disease and cancer, are caused by genetic variations in more than one gene as well as environmental contributions, and obesity is a precursor to these metabolic diseases in the general population.

As reported above, study subjects are currently being enrolled in the SANSCOG project at Kolar district in Karnataka. My work focused on "identifying genetic basis of metabolic disorders and its shared etiology with dementia in Indian population" will be based on the clinical, neurocognitive, anthropometric, and genetic data generated

from this study. We will first check the epidemiological correlations of the complex diseases mentioned in this population. To study the genetic architecture, I will first develop pipeline for accurate and efficient genotype imputation of our study samples from whole genome sequencing data of about hundred individuals from the south India, thus enabling us to include complete information over the genome, deriving haplotype information over untyped genetic variants in our analyses to detect association with traits of interest. Subsequently we would identify the shared genetic architecture of metabolic and neurodegenerative processes by genetic risk scores-based regression models.

Project title: Optimizing Machine Learning algorithms for cognitive impairment classification

Dementia is a clinical syndrome which affected more than 35 million people worldwide in 2010, projected to be 48.1 million people for 2020 with doubling of numbers every 20 years. Alzheimer's disease (AD) represents the primary cause of neurodegenerative dementia. AD pathology is defined by cognitive impairment, behavioral disturbance, and functional disabilities, which have a great impact on the quality of daily life and is major problem for families and caregivers too. It is thus crucial to detect such changes early and to identify the level and the type of impairment in the patients. Neuropsychological assessment plays a crucial role in detecting loss of cognitive functions and change in behavioral and functional state compared to normal conditions. Neuropsychological tests can measure different cognitive domains (e.g., language, learning, and memory) and subdomains (e.g., long-term memory and recognition memory). Identifying the best combination of tests to be used to classify and diagnose AD, is still a matter of debate, and a large amount of subjectivity also lies in the diagnostic process.

Our aim is to use neuropsychological and demographic data to predict cognitively healthy, MCI, AD through the implementation of machine learning models. We also aim to determine a small set of attributes in neuropsychological tests that can be used to reliably diagnose individuals in the community. Our variable selection process would avoid data over-fit, improve classification accuracy, provide faster models, and gain deeper insight into the underlying processes that generate the data.

We had implemented our workflow on a dataset of 1771 individuals from the ROSMAP study by Prof. David Bennett of Rush Univ, Chicago. Each of these 1771 individuals have undertaken total 24 cognitive tests in their baseline visit. We have not yet considered follow up years. The 1771 individuals were divided into 7 sets of training and test data. Each test data included 253 individuals and each individual was tested at least once. The different machine learning algorithms used are as follows: Random forests, Support vector machine, principal component analysis followed by three clustering algorithms- K means clustering, complete and average hierarchical clustering. The

programming software used is R. The preliminary results indicate that all the Alzheimer disease volunteers are predicted correctly except by the hierarchical clustering algorithms. According to the performance of the algorithms on the training data, random forests > support vector machine > K means clustering > complete Hierarchical clustering > average Hierarchical clustering. We then applied Random Forest (RF) with mtry (based on the number of input features) and ntree varying, so as to tune the RF for its best implementation. The multiclass ROC becomes stabilized for the datasets at ntree=800, and mtry=18 for RF. The ROC values for these parameters for the seven datasets are given in the table below. The same table also depicts the multiclass ROC values for Support Vector Machine algorithm with default radial kernel. The table shows that Random forests perform better than Support vector machines for the classification purpose.

	1	2	3	4	5	6	7
SVM	0.8797	0.8823	0.8792	0.862	0.8914	0.8512	0.8639
RF	0.9094	0.939	0.908	0.8633	0.9245	0.9132	0.9203

The healthy and MCI subjects are predicted more accurately than the AD subjects where we have more false negatives than the two former classes.

This is an work in progress. Currently we are devising models to determine a small set of attributes in neuropsychological tests that can be used to reliably diagnose individuals in the community. Ultimately these models will be applied to the Indian population.

2. Dr. Ganesh Chauhan

Project Title: The APOE epsilon4 allele association with mild cognitive impairment/Alzheimer's disease

This project is funded by the Ramalingaswami Fellowship awarded to Dr. Chauhan

Developing countries like India will share the highest burden of dementia in the coming years as by 2030 almost 63% of these cases will reside in low and middle-income countries. Dementia is complex disorder resulting from both genetic and environmental components and their interaction. It is a highly heritable disorder with 60-80% heritability based on studies in twins. Association studies of dementia in particular Alzheimer's disease have identified more than 20 regions in the genome to be associated with risk of dementia. Majority of the work is derived from study on populations of European ancestry and some on African ancestry. A major genetic risk factor for dementia are the epsilon alleles of the gene APOE. Earlier studies have suggested that the allele frequency of the epsilon 4 allele, which is the risk allele for developing dementia is very low compared to other populations of the world. We recently became co-investigators in the "Alzheimer's disease: Understanding Mechanisms for Early Diagnosis and Treatment" project funded by the TATA trust. This project has a longitudinal population based study with sites in

Bengaluru and Hyderabad. Frozen blood for isolation of DNA was available from this project along with phenotyping for dementia, mild cognitive impairment and healthy control status. The lab isolated DNA from the frozen blood for genetic studies.

Highlights

- APOE ε allele genotype status is currently available for 133 subjects (healthy controls, subjects with mild cognitive impairment-MCI and AD) residing in the two South Indian cities of Bengaluru (N=98) and Hyderabad (N=35). Genotype data of 52 subjects have been added since the last year review in 2017.
- The allele frequency of ε4 was 0.15, ε3 was 0.80 and ε2 was 0.05 in these 133 subjects, similar to last year report. After excluding subject with MCI and AD the frequency of epsilon4 was 0.14, epsilon3 was 0.80 and epsilon2 was 0.06.
- The epsilon4 allele was associated with MCI/AD status in subjects from Hyderabad (OR= 11.50, 95%CI=2.06-64.34, P=0.004) and showed a non-significant trend for association with MCI/AD in the subjects from Bengaluru.

Progress

We have Sanger Sequenced 98 subjects for APOE ε allele status from the Bengaluru cohort and 35 from the Hyderabad cohort. Genotyping of a few more subjects from Bengaluru is under way. DNA from 41 subjects from Hyderabad cohort is available with the Bengaluru team but DNA quality of 6 subjects is very bad and PCR amplification cannot be performed.

Genotyping: The ApoE gene region containing the SNPs rs429358 & rs7412 which determine the ApoE allele status was PCR amplified using the primers previously described by Hixson & Vernier.[1] PCR amplified products were purified from the agarose gel and subjected to Sanger Sequencing using the Applied Biosystems platform. The forward primer used for PCR amplification also acted as the sequencing primer.

Genotype calling: Sanger sequencing peaks were aligned and variations were identified using default parameters of the tool novosnp.[2,3] The variation called by novosnp were verified manually after inspecting the sequencing peaks of all individual samples, special attention was paid to heterozygous calls. No discordance was observed between novosnp calling and manual verification.

Sample exclusion in Bangalore cohort: Subjects who are the first or second degree relatives were excluded from association analysis with polymorphisms, the proband or the oldest individual of the family was retained.

Classification of cases and controls: Subjects with MCI and AD were treated as cases while healthy volunteers were treated as controls.

Transformation of quantitative traits: Due to small sample sizes currently quantitative trait data is not being transformed. As many of the quantitative traits did not follow normal distribution (based on histograms and Shapiro-Wilk test) all of them were transformed using the rank based inverse normal transformation so that they can fit into a regression model. The "rntransform" function of the GenABLE package (http://www.genabel.org) was used to do the transformations.

Genotype model: All subjects who are non-carriers of ε4 allele (ε2/ε2, ε2/ε3 & ε3/ε3) were treated as reference while subjects with one or more copy of ε4 allele (ε3/ε4 & ε4/ε4) were treated as variants.

Regression model: Association with MCI+AD was performed using logistic regression. Association with all other traits (cognitive measures and blood biochemistry) were performed using linear regression but only in normal healthy controls. The regression models were adjusted (i) for sex, and age (ii) sex, age and years of education. All association analysis with genotypes was performed using the tool PLINK v 1.9.[4] All association analysis are presented with respect to one or more copy of ε4 alleles.

Meta-analysis: After performing association analysis in the two study cohorts separately, there individual statistics were meta-analyzed using the fixed effect model as implemented in the tool METAL.[5]

Association of AD with years of education and diabetes: Linear and logistic regression were performed in R using the glm function after accounting for sex, age and site for checking association of AD with years of education and diabetes, respectively.

Results:

Hyderabad Cohort							
Frequency cases	Frequency controls	OR	SE	L95	U95	BETA	P-value
0.75	0.2069	11.5	0.8785	2.056	64.34	2.44235	0.00392
Bengaluru Cohort							
Frequency cases	Frequency controls	OR	SE	L95	U95	BETA	P-value
0.2667	0.2838	0.9177	0.4513	0.3789	2.223	-0.0859	1
Meta-analysis							
BETA	SE	P-value	Direction	HetISq	HetChiSq	HetDf	HetPVal
-0.442	0.4014	0.2709	+-	84.7	6.553	1	0.01047

References:

1. Hixson, J.E. & Vernier, D.T. Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhaI. *J Lipid Res* **31**, 545-8 (1990).

2. Weckx, S. et al. novoSNP, a novel computational tool for sequence variation discovery. *Genome Res* **15**, 436-42 (2005).

3. Rijk, P.D. & Del-Favero, J. novoSNP3: variant detection and sequence annotation in resequencing projects. *Methods Mol Biol* **396**, 331-44 (2007).

4. Purcell, S. et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* **81**, 559-75 (2007).

5. Willer, C.J., Li, Y. & Abecasis, G.R. METAL: fast and efficient meta-analysis of genomewide association scans. *Bioinformatics* **26**, 2190-1 (2010).

Conclusions:

- We observe a significant association of APOE epsilon4 status with AD/MCI in the Hyderabad cohort.
- Association of APOE epsilon4 status with AD/MCI in the Bengaluru cohort is not significant due to an imbalance in number of subjects in the case-control group. Recently there has been addition of a large number of cognitively normal subjects in the Bengaluru cohort compared to MCI. This is likely the reason that the significant association with AD/MCI status seen last year in the Bengaluru cohort is not seen anymore.

3. Dr. Smitha Karunakaran

Project title: Early deficits in neural circuit based mechanisms in Mouse Model of Alzheimer's Disease

Overall Goals

The overall goal of this study is to understand the early behavioral deficits during the asymptomatic phase of Alzheimer's Disease (AD) using APP_{Swe}/PS1ΔE9 (APP/PS1) mouse model of AD.

Highlights and Summary

- Deficits in episodic memory retrieval in tasks such as Object-place recognition and Object-in-context recognition were observed in 2m old APP/PS1 mice. However, Novel Object Recognition memory was intact.

- Recall deficits were observed after contextual fear conditioning (cFC) at two months of age in APP/PS1 male mice.

Detailed Progress

Memory decline in the early stages of AD has always been attributed to hippocampal – entorhinal circuit deficits. In terms of the staging of cognitive deficits in AD, it is commonly accepted that episodic memory is first to be impaired, followed by semantic, executive and attention deficits. However, all these studies has been done during the symptomatic phase of the disease pathogenesis. It is getting very evident from recent reports that the disease pathology sets in decades before the symptomatic disease. Therefore, in this study we set out to understand the early deficits with regard to behavior in vivo in mouse model of AD, with special focus on the hippocampus.

In our earlier studies we have demonstrated that 2m old APP/PS1 mice show a significant early impairment in episodic memory tasks such as Object-place recognition and Object-in-context recognition. Further, it was also shown these mice exhibit a significant deficit in associative learning task such as contextual fear conditioning (cFC) at 2 months of age. Both cFc and episodic memory tasks have hippocampus as an important component in there neural circuits. We therefore chose to further understand the role of hippocampus in contributing to early deficits in 2 month old APP/PS1 mice. Therefore, we chose to use the classical hippocampus based paradigm Morris Water Maze (MWM) to check specific hippocampal-dependant learning deficits.

Lesion and selective manipulation studies of the hippocampus has clearly defined a crucial role for hippocampus in spatial learning paradigms such as MWM. Decrease in dendritic spine density in the CA1 region of the hippocampus also refers to spatial learning deficits. Therefore, to further examine whether there are hippocampal dependent spatial learning deficits APP/PS1 mice at an early stage, we performed MWM in this group of animals at 2 months of age. We observed that during the acquisition phase of MWM, there were no differences between 2 month old Wt and Tg mice (Fig. 1A) in navigating and finding the hidden platform (Day1 -6). Though there was a tendency for longer latencies during the initial days, the difference did not reach significance levels. During the probe trial following acquisition, both Wt and Tg mice mice preferentially spent more time in the target quadrant (Fig. 1B). The mice were then taken to the more challenging reverse water maze task, which assess cognitive flexibility and requires the development of new spatial strategy. The position of the platform was reversed or relocated to the opposite quadrant, APP/PS1 Tg mice showed similar escape latencies to Wt control throughout the trial (Day 7-11; Fig. 1A). At the end of the reversal trial, APP/PS1 mice displayed a preference for the new target quadrant (Fig. 1C). These data conclude that APP/PS1 mice show normal spatial learning capabilities, they updated and adjusted their spatial map and search.

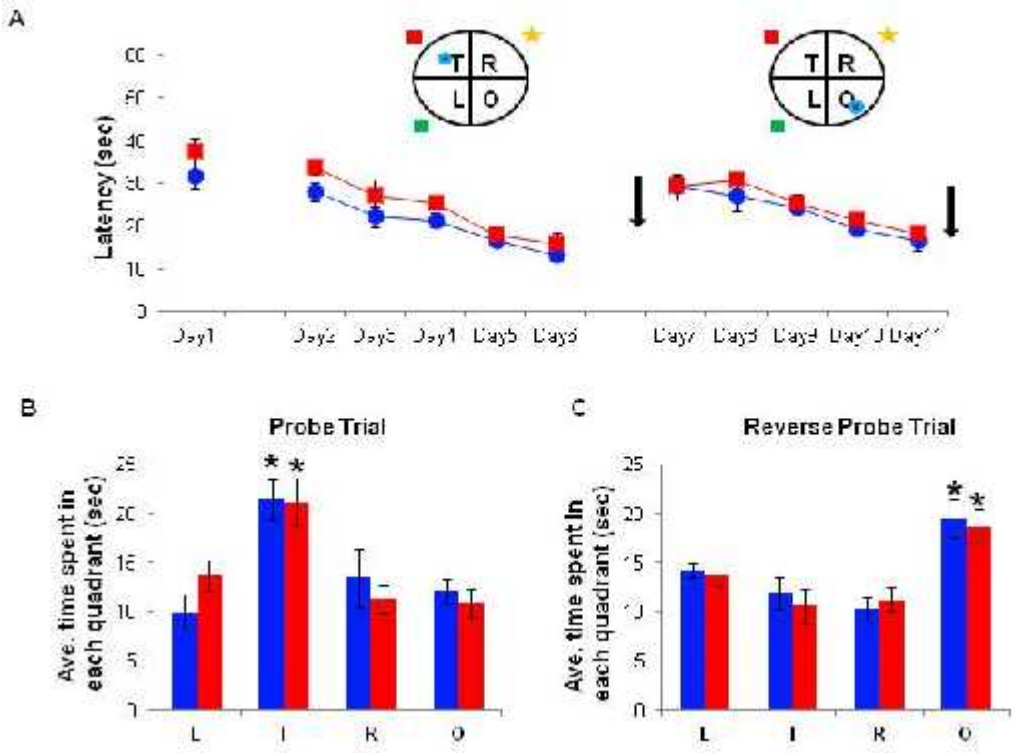


Fig.1 - Evaluation of spatial learning in APP/PS1 mice using the Morris water maze: (A) Differences in latencies between 2m old male wild type (Blue) and APP/PS1 mice (Red) depicted as learning trial trials from Day 2- 6. The first arrow from left indicates the probe trial session conducted at the end of 6th day. The hidden platform was moved to the opposite quadrant (O) during reversal training, followed by the probe trial (second arrow from left). (B, C) Both Wt and APP/PS1 mice showed increase preference for the target quadrant which contained hidden platform. Data are presented as mean ± S.E.M; asterisks denote significant differences.

Conclusion:

- Male APP/PS1 mice early on at 2 months of age do not display spatial learning deficits in Morris Water Maze task but exhibit an aversive learning deficit and episodic-like memory deficits. This denotes the involvement of other parallel memory systems other than hippocampus.

Future Goals:

- Detailed morphological analysis will be done in the medial and lateral entorhinal cortices to determine if the entire entorhinal-hippocampal loop is dysfunctional early on in APP/PS1 mice.

V. The scientific advisory committee of CBR

The scientific advisory committee (SAC) of CBR has been constituted which meets twice a year to review the research protocols at CBR. The committee constitutes of the following members:

1. Prof. Srinath Reddy (PHFI, Delhi), Chairperson
2. Prof. Gangadhar BN (NIMHANS, Bangalore)
3. Dr. Ramesh Hariharan (Strand Life Sciences, Bangalore)
4. Dr. Anurag Agrawal (IGIB, Delhi)
5. Dr. Partha P. Majumder (NIBMG, Kalyani)
6. Prof. Arun Kumar (MRDG-IISc, Bangalore)

So far we had two SAC meetings, on August 28th 2017 and February 2nd 2018.

VI. Academic Collaboration with IISc

In the Governing Board meeting held in March 2017, it was suggested that CBR can take up the joint academic programmes and research with IISc. A proposal on this was sent to IISc and it has approved the following:

- Ph. D students would be allowed to carry out collaborative research at CBR in areas aligned with the mandate and vision of CBR. But the students will be admitted to the Institute and be under the supervision of the Institute faculty. A faculty member from CBR would be the co-supervisor, similar to the ERP Program.
- The scholarship for the students would be provided by the CBR and students may be encouraged to stay outside the institute campus.
- CBR faculty may get involved in offering courses at IISc either individually or jointly with IISc faculty.

Similarly a proposal was sent to IISc indicating that while CBR would equip itself with most of the major research facilities, it would require the usage of some critical facilities from IISc, such as Central Animal facility (CAF), SERC, Library etc. The charges for the usage of these facilities can be paid by CBR.
This proposal also has been approved by IISc

VII. Publications by CBR faculties:

1. Ahmad F, Singh K, Das D, Gowaikar R, Shaw E, Ramachandran A, Rupanagudi KV, Kommaddi RP, Bennett DA, **Ravindranath V**. ROS-mediated loss of synaptic Akt1 signaling leads to deficient activity-dependent protein translation early in Alzheimer's disease. Antioxid Redox Signal. 2017 Dec 1; 27(16): 1269-1280. PMID: 28264587
2. Kommaddi RP, Das D, Karunakaran S, Nanguneri S, Bapat D, Ray A, Shaw E, Bennett DA, Nair D, **Ravindranath V**. J Neurosci. 2018 Jan 31;38(5):1085-1099. PMID: 29246925
3. Protein-altering variants associated with body mass index implicate pathways that control energy intake and expenditure underpinning obesity. Turcot V, Lu Y, …… **Kahali Bratati**, ……, Hirschhorn JN, Loos RJF; CHD Exome+ Consortium; EPIC-CVD Consortium; ExomeBP Consortium; Global Lipids Genetic Consortium; GoT2D Genes Consortium; EPIC InterAct Consortium; INTERVAL Study; ReproGen Consortium; T2D-Genes Consortium; MAGIC Investigators; Understanding Society Scientific Group. **Nat Genet. 2018** Jan;50(1):26-41.

4. Association Between Telomere Length and Risk of Cancer and Non-Neoplastic Diseases: A Mendelian Randomization Study. Telomeres Mendelian Randomization Collaboration, Haycock PC, Burgess S, … **Kahali Bratati**, …. Davey Smith G. **JAMA Oncol. 2017** May 1;3(5):636-651.

5. Bellenguez C, Charbonnier C, Grenier-Boley B, Quenez O, Le Guennec K, Nicolas G, .. **Chauhan G** …et al. Contribution to Alzheimer's disease risk of rare variants in TREM2, SORL1, and ABCA7 in 1779 cases and 1273 controls. Neurobiol Aging. 2017; 59: 220 e1- e9.
6. Christophersen IE, Rienstra M, Roselli C, Yin X, Geelhoed B, Barnard J, .. **Chauhan G** ..et al. Large-scale analyses of common and rare variants identify 12 new loci associated with atrial fibrillation. Nat Genet. 2017; 49(6): 946-52.
7. Rannikmae K, Sivakumaran V, Millar H, Malik R, Anderson CD, Chong M, .. **Chauhan G ..** et al. COL4A2 is associated with lacunar ischemic stroke and deep ICH: Meta-analyses among 21,500 cases and 40,600 controls. Neurology. 2017; 89(17): 1829-39.
8. Wain LV, Vaez A, Jansen R, Joehanes R, van der Most PJ, Erzurumluoglu AM, .. **Chauhan G** .. et al. Novel Blood Pressure Locus and Gene Discovery Using Genome-Wide Association Study and Expression Data Sets From Blood and the Kidney. Hypertension. 2017.
9. Duperron MG, Tzourio C, Sargurupremraj M, Mazoyer B, Soumare A, Schilling S, **Chauhan G**, et al. Burden of Dilated Perivascular Spaces, an Emerging Marker of Cerebral Small Vessel Disease, Is Highly Heritable. Stroke. 2018; 49(2): 282-7.
10. Kommaddi RP, Das D, **Karunakaran S**, Nanguneri S, Bapat D, Ray A, Shaw E, Bennett DA, Nair D, Ravindranath V. Ab mediates F-actin disassembly in dendritic spines leading to cognitive deficits in Alzheimer's disease. J Neurosci. 2018. Jan 31;38(5):1085-1099.

VIII. Invited talks and Presentations:

1. Prof. Vijayalakshmi Ravindranath gave the talk titled "The Degenerating brain: mechanisms of therapy" at IBRO Workshop BHU (April, 2017)

2. Prof. Vijayalakshmi Ravindranath gave the talk titled "Aging Population and the Challenge for India"at the International Summit on Aging, University of Wisconsin, Madison (April, 18-19 2017)

3. Prof. Vijayalakshmi Ravindranath gave the talk titled "The disappearting Synapse in Alzheimer's disease" at Colorado State University, Boulder (April, 25 2017)

4. Prof. Vijayalakshmi Ravindranath gave the talk titled "The Changing Demography of India and Challenge for Neuroscience" at M.S.Ramaiah College of Medicine (June, 20 2017)

5. Prof. Vijayalakshmi Ravindranath gave the talk titled "The Changing Demography of India and Challenge for Neuroscience"at CDRI Foundation Day lecture (Sept, 22 2017)

6. Prof. Vijayalakshmi Ravindranath gave the talk titled "The Changing Demography of India and Challenge for Neuroscience" at the NASI Annual meeting (Dec, 8 2017)

7. Dr. Bratati Kahali was invited to deliver a talk at the 13th Indo-Australian Biotechnology Conference OMICS IN HEALTH on 30-31 October 2017 in Brisbane, Australia.

8. Dr. Bratati Kahali was invited to deliver a talk at the IISc- CMC conclave on 8- 9 January 2018 in IISc, India.

9. Dr. Ganesh Chauhan was invited to give a talk "Genetic risk factors for stroke and cerebral small vessel disease" in the Neuroscience 2017 Science academies Lecture workshop on Recent advances in research on brain & diseases organised by M.S. Ramaiah Medical College, Bengaluru, on21st June, 2017.

10. Dr. Ganesh Chauhan was invited to give a talk ""Understanding Genetic Basis of Familial and Sporadic diseases" at ESIC Medical College, Bengaluru on 27th October, 2017

11. Dr. Ganesh Chauhan was invited to give a talk "Bioinformatics in the study of complex diseases" at Institute for Communicative and Cognitive Neuro Sciences [ICCONS], Shoranur, Kerala 12-13th January, 2018

12. Dr. Ganesh Chauhan was invited to give a talk at IBRO-APRC-Associate School of Neuroscience Workshop on "Recent Advances in Tools and Techniques in Neuroscience Research" organized by Department of Biotechnology Savitribai Phule Pune University, Pune, MAHARASHTRA (26-31 March, 2018). Title of talk "Neuro-Omics".

13. **Karunakaran S,** Kommaddi RP, Das D, Ray A, Bennett DA, Ravindranath V. Synaptosomal F-actin loss mediates early behavioural deficits in Alzheimer's Disease mouse model- Presented at the Society for Neuroscience-2017 Annual Meeting held in Washington DC, USA, November 11-15, 2017. – Poster Presentation

14. Kommaddi RP, **Karunakaran S**, Ravindranath V. Synaptosomal F-actin loss occurs early in Alzheimer's disease mouse model and leads to contextual fear conditioning (cFC) behavioral deficits – Presented at The Alzheimer's Association International Conference-2017 held in London, UK, July 16-20, 2017. – Poster Presentation

IX. Awards

Prof. Vijayalakshmi Ravindranath was given the following awards:

i. Jawaharlal Nehru Birth Centenary Lecture Award, INSA

ii. Elected Member Dana Alliance for Brain Initiatives, USA

iii. Shree Vanamali Seva Prashasti Award 2017

iv. Travel Award, Society of Free Radical Biology & Medicine (awarded to her PhD student Aditi Verma)

Dr. Ganesh Chauhan and Dr. Bratati Kahali have been awarded the Ramalingaswami Fellowship, by DBT.

X. Lectures:

Under the CBR lecture series the following lectures were delivered:

1. Lecture on "Biologic Architecture of Age-Related Cognitive Decline" by Prof. David A. Bennett, Director of the Rush Alzheimer's Disease Center and the Robert C. Borwell Professor of Neurological Sciences at Rush University Medical Center, Chicago.

2. Lecture on "**Molecular neurobiology of Alzheimer's disease" by Prof. Sangram S. Sisodia,** Department of Neurobiology, The University of Chicago, Chicago, USA, is the Thomas Reynolds Sr. Family Professor of Neurosciences and Director of the Center for Molecular Neurobiology at The University of Chicago.

3. Talk by **Mr. Kris Gopalakrishnan** on "**Why brain research is important for us, in India".**

4. Lecture on **"Research across Borders**" by **Prof. Subra Suresh**, President Carnegie Mellon University, USA.

5. Lecture on "**Using Machine Learning to Study Neural Representations of Language Meaning"** By **Prof. Tom M. Mitchell**, Fredkin University Professor at Carnegie Mellon University, USA.

6. Lecture on "**Enjoying a three-way marriage: A story of Maths, Biology, and Medicine" by Prof. ANURAG AGRAWAL** Principal Scientist at CSIR-Institute of Genomics & Integrative Biology, New Delhi.

XI. List of Faculty and Staff Members

Convener:
1) Prof.Vijayalakshmi Ravindranath

Faculty:
1) Dr.Ganesh Chauhan
2) Dr.Bratati Kahali
3) Dr.Smita Karunakaran

Adjunct Faculty:
1) Prof. Y Narahari,IISc
2) Prof. G Rangarajan,IISc
3) Prof.H P Khincha
4) Prof.Sridharan Devarajan,IISc
5) Prof.Vijayalakshmi Ravindranath
6) Prof. Arun Kumar,IISc

Visiting Faculty:
1) Dr. P T Sivakumar,NIMHANS
2) Dr.Naren P Rao,NIMHANS
3) Dr.Ganeshan Venkatasubramanian,NIMHANS
4) Dr.Girish N Rao
5) Prof.Gaiti Hasan,NCBS

Technical Staff:
1) Dr.Khader Valli Rupanagudi

Post-Doctoral Fellow:
1) Dr. Devendra Meena
2) Dr. Santi Natarajan

Administrative Staff:

1) Mr.M L V Subramaniam — Upto 30th September 2017
2) Mr.S Rajagopalan — Upto 30th September 2017
3) Mrs. P K Bharathy — Upto 30th September 2017
4) Mr.R.Mohan Das
5) Ms.Aruna Poojary
6) Mrs.Sudha Srikanth

Project Staff:

1. Mrs.Diji Kuriakose
2. Mr.C.Mohana
3. Ms.Krithika Subramanian
4. Dr. Ammu Lukose
5. Dr. Manasi Oza
6. Dr. K.S.Harsha
7. Dr. Aneelraj
8. Dr. Niranjanagowda K A
9. Dr. Abhinav Kumar — Upto Feb 2018
10. Dr.Lakshmi Sowjanya — Upto Feb 2018
11. Ms. Anu K.N
12. Ms. Shreya Jha
13. Mr. K.V.Rahul
14. Mr.Shivananda K S
15. Mr.Harikrishna G
16. Mr.Ramesh K
17. Mr.Rajesh
18. Ms.Mino Susan Joseph
19. Ms.Gayathri
20. Mr.V.N.Chandrasekhar