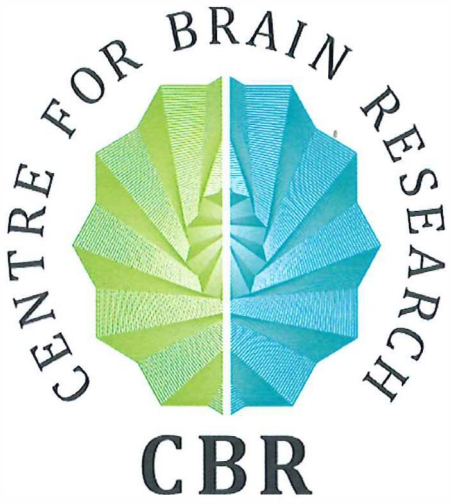
Centre for Brain Research

An autonomous Centre at the Indian Institute of Science



Annual Report 2016-2017

Mandate and Vision

- Carry out basic research to understand brain function in health and disease.
- Foster focused research programs for inter-disciplinary neuroscience research to discover rational therapies for dementia and other age-related brain disorders.
- Networking of neuroscientists among institutions across the country, with emphasis on neurodegenerative disorders.

The human brain mediates the perception of the world around us, generates the myriad thoughts, memories, and emotions that make us uniquely human, and controls our immediate and long-term behavioral responses to the environment.

Research at CBR represents an integrative approach encompassing genetics, imaging, molecular, functional, computational methods while bringing together large groups with diverse expertise to address the complex challenges of understanding brain functioning in health and disease. We aim to develop and foster large scale research programs and build capacity focused at inter-disciplinary neuroscience research in India, thereby setting the stage for our scientists to contribute significantly to the endeavor of understanding the human brain and discovering rational therapies and cures for brain disorders, in addition to gaining insight about the complex cognitive and behavioral functions executed by the brain. Alzheimer's disease (AD), one of the most common forms of dementia, is a devastating condition of memory loss and cognitive dysfunction, mood swings, and behavioral changes leading to progressive decline in the quality of life until death. Age is a major risk factor for AD and other neurological disorders, most of which are ill-understood and many defy current therapeutic strategies. Considering the enormous societal and economic burden posed by AD, it is imperative to understand the normal aging process in the brain, which often results in decline of specific cognitive functions, variably across individuals. This will aid us in discovering methods for early diagnosis and intervention strategies to slow down the progression of the disease and delay dementia.

CBR is funded by Pratiksha Trust (set up by Mr. Kris Gopalakrishnan, co-founder of Infosys and Mrs. Sudha Gopalakrishnan). This is a unique initiative in the current research environment in India, wherein, conventionally, most of the academic research has been carried out through public funding. The Centre's private endowment offers unprecedented flexibility in faculty and staff recruitment and operation that is necessary for large-scale scientific endeavor. The Centre also received a generous endowment from ex-IAS officer Ms. Sharwaree Gokhale who passed away on 15th January 2016. In her will, Ms. Gokhale donated a major portion of her estate to the centre. Her contribution could help progress our understanding of the human brain. Another philanthropist Mr. Prashant Bharat Wani from Nashik, in memory of his father who died due to brain stroke, has given a generous donation to CBR. CBR is immensely thankful for the philanthropic support and aims to build capacity for interdisciplinary neuroscience research in India.

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 is ongoing with architects and the construction work should commence in the coming months. CBR has an Advisory Board (chaired by Nobel

Laureate, Torsten Wiesel) and a Governing Board (chaired by Director, IISc), drawing inputs from the best minds in India and abroad.

Research activities at CBR

- **Srinivaspura Aging Neuro Senescence and COGnition (SANSCOG)**
  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), Prof. G Rangarajan (IISc), Prof. Y Narahari (IISc) among others.
  CBR, in collaboration with National Institute of Mental Health and Neurosciences (NIMHANS) and the Indian Institute of Science (IISc) has started the Srinivaspura Aging Neuro Senescence and COGnition (SANSCOG) study, which is a large-scale prospective community based cohort study with planned 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. The study cohort (n=10,000) would comprise of cognitively healthy individuals without dementia aged 45 years and above. The cohort will be evaluated with detailed clinical, neurocognitive, lifestyle, anthropometric, biochemical and genetic assessments in the baseline and during periodic follow up. A subsample of the study cohort will be evaluated with advanced high-throughput genetic and multi-modal neuroimaging measures. This study will generate a database comprising of genetic, biochemical, clinical, neuroimaging and neurocognitive data that can help in further exploration of the pathophysiology of normal and pathological aging using systems biology based approaches. The pilot phase of the study is proposed for 2 years. Prospective population based longitudinal aging study with periodic monitoring of cognitive function, risk factors, protective factors and biomarkers for Alzheimer's disease (AD) is an important approach to understand the pathophysiology of cognitive ageing and AD. Genetic and other environmental risk factors for AD are likely to vary across populations. Indian research studies in this area are not available. Thus, we have undertaken this study to identify the risk and protective factors for cognitive ageing and dementia in the Indian population; in this process, we will assess how the risk factors for metabolic disorders, including cardio-vascular and diabetes mellitus correlate with risk of cognitive ageing and dementia in the Indian population; and how amyloid and Tau imaging biomarker correlate with cognitive function in Indian population. We will process all the data obtained from epidemiological survey, anthropometric measurements, cognitive assessments, clinical and biochemical analyses to form a comprehensive collection of information that is organized so that it can easily be accessed, managed, and updated. This will be achieved by ensuring seamless transfer of epidemiological, anthropometric, clinical, biochemical and cognitive assessment results to a relational database system at CBR, which individual investigators can query according to their research needs, which permits the participation of the larger scientific community in data analysis, especially of the imaging and genetics data. We have also embarked upon the task of digitizing the cognitive questionnaire to be exercised on our study individuals, which will ensure efficient flow of data into our databases and importantly, this questionnaire can be used by similar projects across the country.

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. Shri Devraj Urs Medical College, Kolar are also collaborative partners in this project.

The individual research focus of Dr. Bratati Kahali under this project is provided below.
Project title: Decoding genetic basis of metabolic disorders and its shared etiology with dementia in Indian population
Investigator: Dr. Bratati Kahali
Polygenic and complex human disease like, dyslipidemia, type 2 diabetes (T2D), cardiovascular disease, 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. My previous publications have reported more than hundred regions in the human genome wherein variations are associated with genetic predisposition to obesity in Europeans, and we provide strong support that central nervous system genes and pathways are responsible for the aforementioned genetic susceptibilities. Large scale comprehensive genetic analyses for such complex disorders are lacking in India.
To this end, we have set up prospective community based cohort studies with long term follow-up over many years for comprehensive evaluation of normal age-related disorders in Bangalore urban and Kolar district in Karnataka. The study cohorts (N=10,000) comprising of cognitively healthy individuals in the age group 45 years and above will be evaluated with detailed clinical, neurocognitive, lifestyle, anthropometric, biochemical and genetic assessments in the baseline and during periodic follow up. 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 and anthropometric data generated from this study. To accomplish this goal, I will first develop pipeline for accurate and efficient genotype imputation of our study samples from whole genome sequencing data of several 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. This project has been submitted for the Ramalingaswami Fellowship application for 2017.

- **GenomeIndia**
  Investigators: Dr. Bratati Kahali (CBR), Dr. Ganesh Chauhan (CBR), Prof. Vijayalakshmi Ravindranath among others.
  CBR co-ordinated the launch of GenomeIndia initiative – a first of its kind in the country which will work towards having a reference human genome having adequate representation of the diverse Indian population. Director, IISc formally launched the project. The meeting was attended by heads of institutions or their appointed representatives from across the country. DG, ICMR and Secretary, DBT had also shared their views in the meeting. The availability of a comprehensive catalog of genetic variations from India will serve the following 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 1000 individuals with the aim of mapping the haplotype structure and genetic variation in this Indian population.

- **"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.

  Project Title: The APOE epsilon4 allele association with mild cognitive impairment/Alzheimer's disease
  Investigator: Dr. Ganesh 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.
  The lab Sanger sequenced 56 subjects for APOE ε allele status from the Bangalore site (AD=1, MCI=8, healthy controls=47). Additional APOE ε allele status for 25 subjects (AD=3, healthy controls=23) was obtained from the Hyderabad site. 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. 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. Sanger sequencing peaks were aligned and variations were identified using default parameters of the tool novosnp. 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.
  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. Subjects with MCI and AD were treated as cases while healthy volunteers were treated as controls. 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 GenABEL package (http://www.genabel.org) was used to do the transformations.

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. 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. All association analysis are presented with respect to one or more copy of ε4 alleles. 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. 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.

The allele frequency of epsilon4 was 0.13, epsilon3 was 0.85 and epsilon2 was 0.02 in this South Indian study population. The number of years of education showed a protective trend for association with MCI/AD status (beta=–2.47, SE=1.97, P=0.213). Carriers of the epsilon4 allele were at a higher risk for MCI/AD (OR= 8.81, 95%CI=1.31-59.06, P=0.025, association statistics adjusted for age and sex). Association with MCI/AD became stronger after accounting for years of education (OR= 14.13, 95%CI=1.53-130.74, P=0.0196). There was little heterogeneity for association statistics between the two sites ($P_{heterogenity}$= 0.79). We did not observe association with measures of cognition in this small sample set. The epsilon4 allele of APOE was associated with MCI/AD in this South Indian study population. This part of the work was presented at the AAIC 2017 meeting held in London as an abstract titled "The APOE epsilon4 allele is associated with mild cognitive impairment/Alzheimer's disease status in South Indians".

Project title:
Comparative analysis of different methods of supervised and unsupervised classification of a given cognitive score data
Investigator: Dr. Bratati Kahali

Given a dataset of cognitive test encompassing logical memory, visuo-spatial ability, attention and other related aspects, we are applying different algorithms and infer which of them correctly predict the clinical diagnoses of Alzheimer's, mild cognitive impairment, and healthy. This includes 39 tests executed on each of 69 volunteers. This dataset is from the TATA trusts funded project.

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 are currently working on building the models taking into account the correlation between individual test measures.

In a nutshell, CBR is actively working towards its goal of fostering focused research programmes to build capacity for inter-disciplinary neuroscience research in India: the primary goal of which will be to discover rational therapies for dementia with state-of-the-art approaches.

LIST OF STAFF MEMBERS

FACULTY:

1. Dr. Ganesh Chauhan
2. Dr. Bratati Kahali

ADJUNCT FACULTY:

1. Professor Y. Narahari, IISc.
2. Professor G. Rangarajan, IISc.
3. Professor Vijayalakshmi Ravindranath, IISc.
4. Professor H.P. Khincha
5. Professor Sridharan Devarajan, IISc.

VISITING FACULTY:

1. Dr. P.T. Sivakumar, NIMHANS
2. Dr. Naren P Rao, NIMHANS
3. Dr. Ganesan Venkatasubramanian, NIMHANS

ADMINISTRATIVE STAFF:

1. Mr. M.L.V. Subramaniam
2. Mr. S. Rajagopalan
3. Mrs. P.K. Bharathy
4. Mr. Mohana
5. Mrs. Diji Kuriakose

LAB HELPER/ATTENDER

1. Mr. S VISHWANATH
1. Mrs. Lakshmamma

Publications:

1. Rare and low-frequency coding variants alter human adult height.
   Marouli E, Graff M,..., ***Bratati Kahali****..,et al. paper from GIANT consortium.
   Nature, 542(7640):186-190, 2017. [* pending work from postdoctoral lab in Univ of Michigan, USA]

2. The association between genetically elevated telomere length and risk of cancer and non- neoplastic diseases.
   Haycock P, Burgess S, ..., ***Bratati Kahali*** *,...., et al.
   JAMA Oncology, 3(5), 636-651, 2017. [* pending work from postdoctoral lab in Univ of Michigan, USA]

3. Ahmad F, Singh K, Das D, Gowaikar R, Shaw, E, Ramachandran A, Rupanagudi KV, Kommaddi RP, Bennett DA and Ravindranath V: ROS-mediated loss of synaptic Akt1 signaling leads to deficient activity-dependent protein translation early in Alzheimer's disease. **Antioxid Redox Signal** doi: 10.1089/ars.2016.6860.

Presentations:

1. Dr. Bratati Kahali was invited to participate in the "One-day UK-India workshop on precision medicine" organized by the British Deputy High commission on Nov 9, 2016.
2. Dr. Ganesh Chauhan was invited to give a talk "Genetic risk factors of structural and vascular measures of neurodegeneration" in the Conference on Genes, Neurodevelopment and Neurodegeneration organised by Centre For Human Genetics, Bengaluru on 26th August, 2016.
3. Dr. Ganesh Chauhan was invited to give a talk "Introduction to Genetic Epidemiology: Heritability, GWAS, Transethnic GWAS" in the "NEUREPIOMICS, a summer school on "Epidemiology of vascular and brain aging in cohorts with large scale imaging and omics data" organised by University of Boston and the BROAD Institute on 23rd September, 2016.
4. Dr. Ganesh Chauhan was invited to give a talk "GWAS identify FOXF2 as a novel locus for stroke and cerebral small vessel disease" in the Strand Life Sciences organised by Strand Life Sciences Pvt Ltd, Bengaluru on 6th October, 2016.
5. Dr. Ganesh Chauhan was invited to give a talk "FOXF2, a novel risk locus for stroke and small artery World Stroke Congress-2016 disease: a genome-wide association study" in the World Stroke Congress-2016 organised by Hyderabad International Convention Centre (HICC), Hyderbad on 27 th October, 2016.
6. Dr. Ganesh Chauhan was invited to give a talk "FOXF2, a locus for stroke and small artery disease" in the International Stroke Genetics Consortium meeting-2016 held at Milan on 3rd November, 2016.
7. Dr. Ganesh Chauhan was invited to give a talk "Genetic risk factors for stroke" in the ARDSI 20th Annual National Conference, organised by All India Institute of Speech and Hearing, Mysore on 10th December, 2016.
8. 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.

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.