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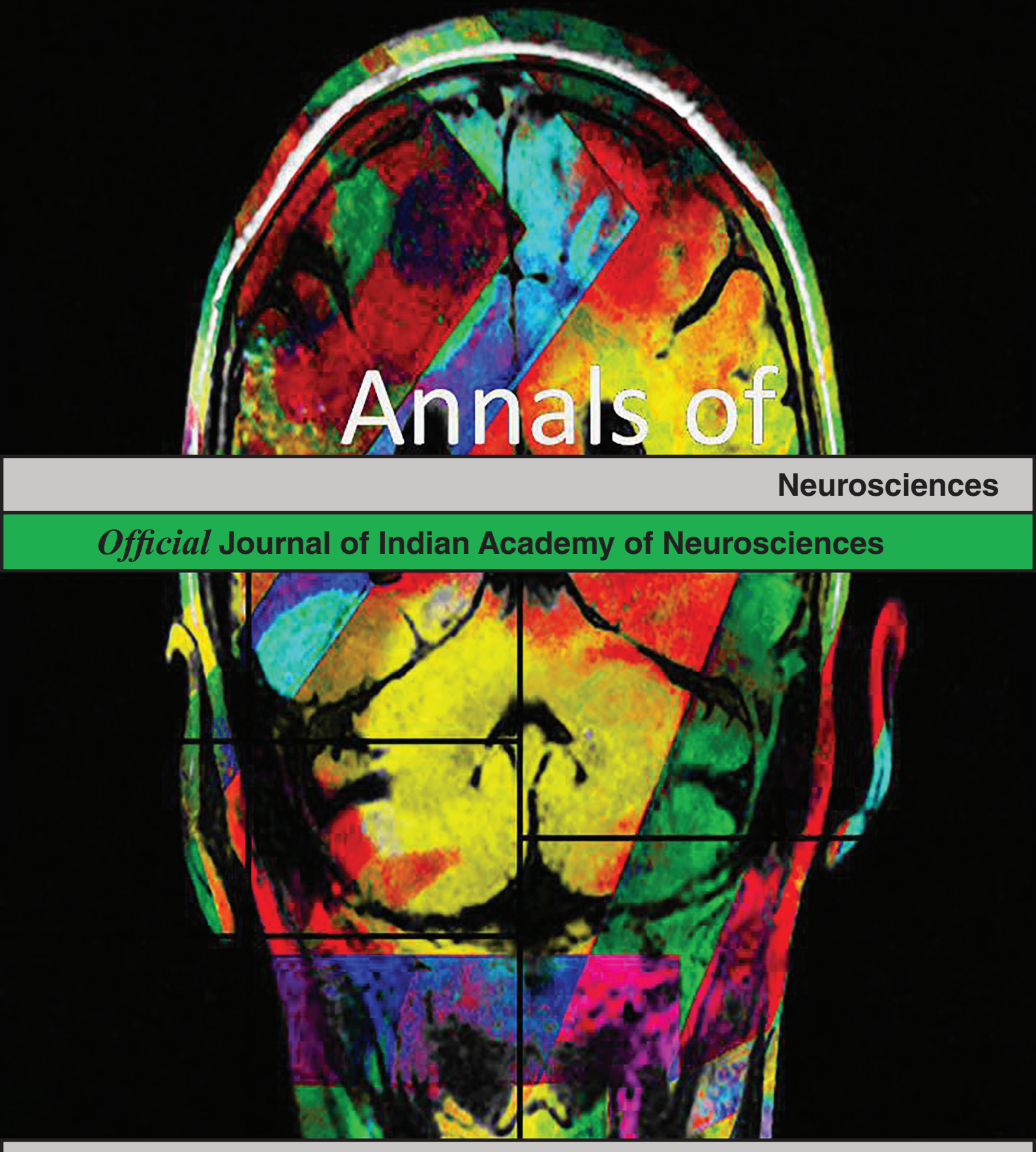

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The Madras Medical Mission
"Compassionate Quality Healthcare"

3rd Madras Medical Mission Genetics Meeting 2018

September 7–9, 2018, Chennai, India

Guest Editors
Bibhas Kar, Chennai
Mitali Mukerji, Delhi
C. R. Srikumari Srisailapathy, Chennai

Editorial Assistant
Priya Mehra, Chandigarh

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3rd Madras Medical Mission Genetics Meeting 2018
Symposium on 'Advances in Genetic Diagnosis of Neurological Disorders'

Editorial

Advancements in Neurogenetics and Genomics

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The 3rd Madras Medical Mission Genetics Meeting 2018 comprising of a symposium on 'Advances in Genetic Diagnosis of Neurological Disorders' and two-day hands-on workshop on 'Exome Sequence Analysis and Interpretation' held from September 7th to 9th, 2018 was an event organised jointly by the MMM – Center for Genetic Studies & Research, Chennai and CSIR – Institute of Genomics and Integrative Biology, Delhi. This marks the Department's third year of holding conferences dedicated to the genetic aspect of a singular medical field.

The theme of symposium was very timely as genetics is not confined to one branch of medicine but is multidisciplinary and involves all branches of medicine. Neurogenetics is one such branch which has rapidly risen as a field that is likely to significantly help us solve the mysteries of the brain with the application of advanced molecular genetics and genomic techniques. It is the study of the genes on a molecular level to understand the functioning of the nervous system. Many neurological diagnoses, especially in children, are genetic in nature, with a mutation leading to production of an abnormally functioning protein that gets expressed in the diseased nervous system. Hence, the results of our efforts to combat neurological diseases will depend on how successful we are at neurogenetics and genomics in the years to come. The rapid identification and characterization of genes of neurological relevance holds great potential for offering insight into the diagnosis, management, and understanding of the pathophysiology of neurological diseases. Therefore, there is a crucial need to employ genetic techniques to understand the complexities involved in neurological disorders.

Genetics and Genomics research provides hope for those who have been affected by neurogenetic diseases. In the era of "omics", we have a potentially unlimited ability to obtain incredible amounts of information on the myriad of factors that are involved in the clinical outcome of neurogenetic diseases. In today's world not only do we understand the genetic basis of many neurological disorders well, but we are also making rapid progress towards modulating gene function to alleviate or treat afflictions. Exome sequencing is a technique that is dominating the genetics field with its understanding of the protein encoding regions of the genome. It is especially valuable in finding a mutation in a known gene with an atypical phenotype, in identifying novel gene mutations and in identifying incomplete penetrance. Although exome sequencing is faster and more cost effective than compared to whole genome sequencing (WGS) it still has its disadvantages which are covered by WGS which generates a more uniform coverage of the genome taking advantage of longer reads thereby allowing for better determination of rearrangements, copy number variations and other structural variations.

The presentations and research papers received conform to the theme of neurogenetics and genomics. These abstracts are an amalgamation of clinical and basic neurogenetics topics to be included in this issue of the journal. It involves a variety of subjects from basics of genetics and genomics medicine, next generation sequencing, cytogenetic microarray to neuromuscular diseases, dystrophies, ataxias, neurodegenerative diseases, channelopathies, epilepsy, aneurysm, stroke, Parkinson's disease, Alzheimer's disease, along with selected abstracts of young researchers.

We feel privileged and honoured to be a part of this special issue of Annals of Neurosciences that highlights the neurogenetic and genomics science presentations during this conference.

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3rd Madras Medical Mission Genetics Meeting 2018
Symposium on 'Advances in Genetic Diagnosis of Neurological Disorders'

Invited Lectures

Introduction to Genomics Medicine: Genome & Exome Sequencing for Rare Genetic Diseases

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One of the major advances that happened in the last decade has been the availability of high-throughput approaches for DNA sequencing which has seen tremendous applications in healthcare and research. This has been largely possible with the advent of newer sequencing approaches which offer a higher throughput, lower cost and faster turnaround times, and appropriate computational tools to enable the processing and analysis of information. On one end, this technology enables to elucidate the genomes of individuals and how genomic variations could influence the life of the individual, while on the other side, it enables one to probe into the pathophysiology of diseases and have a mechanistic view of the disease processes. It is widely believed that these advances would be immensely useful in healthcare through Predictive, Preventive, Precise, Personalised and Participatory Medicine. In the present talk, we would describe our experiences with the use of genomics approaches in Rare Genetic Diseases. The talk would also discuss the way forward, detailing the ongoing initiatives including the GUaRDIAN Consortium involving a large clinical network working on Rare Genetic Diseases in India.

Next Generation Sequencing for Mitochondrial Disorders – Implications for Neurological Diseases

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The availability of the human genome reference sequence has opened opportunities for a new era of genomic medicine. This coupled with the significant advancements in the genome sequencing technologies have now made it possible to sequence the genomes of individuals in a much shorter time span and at an affordable cost. One of the major applications of such genomic technologies in the clinical settings is in the identification and annotation of variants associated with rare genetic diseases. It has been estimated from various projections that India is home to over 70 million people suffering from a genetic disease. Mitochondrial disorders contribute to a significant proportion of the genetic diseases. It is estimated that approximately 1 in 5000 individuals suffer from a mitochondrial disorder. Individuals with mitochondrial disorders have a very wide clinical presentation with significant neurological involvement. We will share our experiences from the Genomics for Understanding Rare genetic Diseases India Alliance Network (GUaRDIAN) and demonstrate the power of genomics for systematic characterization and diagnosis of mitochondrial disorders with significant neurological defects.

Clinical Utility of NGS and Cytogenetic Microarray (CMA) in Intellectual Disability (ID)

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Intellectual disability is the significant subnormal intellectual functioning with deficits in adaptive behaviour and manifestation during developmental period (IQ<70). Often the term 'Developmental Delay' is used for children below 5 years of age with mental and motor delay (DQ) in whom formal IQ testing is not possible. The precise confirmed diagnosis of ID is a clinical challenge which is most important in genetic counselling and prenatal diagnosis in the affected families. Despite the recent advances in laboratory diagnostic techniques, 30-50% of the ID cases remain unidentified. However, the 21st century has opened the new era of genomic technology like cytogenetic microarray (CMA) & next generation sequencing (NGS) platforms in clinical practice which offer the in-depth details about genes involved and clinically correlated mutations, e.g. microdeletions, duplications, inversion, insertions, subtelomeric abnormalities and single nucleotide changes (SNP). The powerful bioinformatics tools coupled with NGS and CMA technology has led to the discovery of various unknown causes of ID. The CMA is a high-resolution chromosomal technique detecting gains or losses of smaller than 10 kb size and has proven to be of significant utility in the diagnosis of patients with ID / with or without multiple congenital anomalies (MCA) and autism spectrum disorder (ASD). Hence, ACMG has recommended CMA as a first–tier test in the genetic evaluation of children with ID. Similarly NGS is the massively parallel sequencing method used with a cost-effective approach & efficacy to screen large number of genes & their intronic or exonic changes correlating with clinical

phenotypes in short time with more reliability. The NGS is often use when clinically suspected target gene involvement is more definite. It is now feasible to trace the genomic etiology with biomarker in hand for evaluating the recurrence risk of the disease which allows future pregnancy management and prenatal genomic diagnosis in the affected family. The prenatal microarray or NGS test can detect genetic abnormalities in foetus as well and is most beneficial when ultrasound sonography identifies structural anomalies or dysmorphism in the foetus. The various reproductive options for prevention of ID can thus be made available to the parents using latest genomic technologies. As the ID has different genetic etiology ranging from single gene disorder (IEM) to complex genetic rearrangements (ID with epilepsy), the option for CMA and/or NGS is decided by an experienced clinical or medical geneticist to arrive at the proper phenotype – genotype correlation and ascertain the precise cause of the patient with ID. The various ID cases with MCA/ASD will be illustrated to emphasis the clinical utility of NGS and CMA technology in the diagnosis, prevention & genetic counselling.

Utility of Genetic Testing in Children with Neuromuscular Disorders

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The availability and research of the newer diagnostic techniques are ever increasing in our country and that has also helped to bring down costs and make it within the reach of the common man. This has also meant that the doctors who see these patients have to keep up to date with the available testing methods and how to utilize them for a given patient. The other aspect to this would be to understand that these genetic testing reports not only help in making a more precise diagnosis for the index case but also in many other ways like counseling the family, carrier screening for the immediate family / close relatives, prenatal diagnosis during the next pregnancy etc. The most exciting advance over the last decade is the utility of the genetic reports in targeted therapies particularly for neuromuscular disorders. It would also be important to understand that the genetic background the child does play an important role in the response of the certain therapies including use of drugs. The dosage of drugs also varies quite widely sometimes depending on which racial background one belongs to particularly with respect to neuromuscular disorders. There are also some geographical differences in the incidence / prevalence in the genetic mutations in our country and the possible reasons for the same have to be identified so that we can look at prevention if we can. National registries will help address these issues as the genetic variations and the prevalence / incidence of these common NMD's will become much clearer. Such statistics will also help us to seek government support / help in not just spreading awareness but also looking at preventive strategies. Capture of data such as this would also help in deciding the Governmental budget allocation and support strategies. A registry at national level would also bring in interest from both national and international agencies who are seeking to do research in to certain aspects including newer treatments for these disorders. Over the last decade in particular a number of newer treatments have come through including genetic modifying therapies and for all this to happen in our country we need to have good statistics as a base. It is also true that our families cannot afford to travel to other countries for newer therapies and so research in to common NMD's which are progressive and shortens the life span of these children need to happen in our country where we have such good clinicians / brilliant researchers / basic scientists / facilities. Registries such as this will help us all to get together work as a team to find solace / cure many of these children / adults suffering from progressive / debilitating forms of NMD's.

Spinal Muscular Atrophy, the Exciting Journey from Disease to Therapy Heralds a New Era in Neurogenetics

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The understanding of the molecular structure of the SMN 1 and 2 genes led to accurate diagnostic and carrier tests. The role of SMN2 gene in modifying the clinical severity of SMA was soon clarified. The demonstration of the molecular differences in the structure of SMN1 and SMN2 genes led to the discovery of the therapeutic role of antisense oligonucleotides and the approval of Neusinersen as drug, both in USA and Europe. Simultaneously there have been reports of successful trial of gene therapy in humans for this disorder.

We will present our 20-year-experience in diagnosis of this disorder in 2077 suspected cases of SMA, with a positive rate of 80%. We will also describe the results of 950 prenatal diagnoses that we carried out. As this disorder is a severe autosomal recessive disorder contributing significantly to infant mortality we carried out a study on the carrier frequency in the Indian population, and this will be outlined. The results of therapy with Nusinersen (anti-oligonucleotides) and gene therapy for this devastating disease will be summarized. The promises of this success story in heralding the dawn of a new era in neurogenetics will be highlighted.

Muscular Dystrophies in Genomic Era – Strategies in Management

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This is a group of genetic disorders which primarily affects the muscle. The cause is heterogeneous, and all types of inheritance patterns are seen which vary from X linked to autosomal recessive and autosomal dominant types. The age of involvement varies from congenital to late adult onset. The commonest of all muscular dystrophies are dystrophinopathies which include Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD). DMD and BMD are X-linked recessive disorders affecting mainly males. The rest of the limb girdle muscular dystrophies (LGMD) together comprise a similar kind of phenotype due to involvement of the carious muscle and membrane proteins. The overlapping phenotype often makes clinical diagnosis of limb girdle muscular dystrophies challenging for a clinician. Genetic abnormalities for the majority of the LGMD and congenital muscular dystrophies are now well delineated. Next generation sequencing has provided an accurate non-invasive method of diagnosis where uncertainties associated with more conventional diagnostic methods such as muscle biopsy have been minimized. It has also bought surprises with diagnosis of metabolic disorders which can mimic LGMD such as Pompe disease and glycogen storage disease type III leading to better management of the potentially treatable conditions. Other LGMD such as Facioscapulohumeral dystrophy (FSHD), Sarcoglycanopathies, dysferrlinopathy, GNE related myopathies follow dystrophinopathies. Majority of them need multidisciplinary supportive treatment in a setting of genetic counselling and prenatal diagnosis. However, progress has been made in DMD as well as Pompe disease with newer trials of exon skipping and enzyme replacement therapy respectively becoming successful.

Cerebellar Ataxias in the Indian Population: from Research to Translational Applications

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Cerebellar ataxias are a group of rare progressive neurodegenerative disorders whose prevalence is on an average 4/10000 individual. These diseases could be inherited or can be sporadic. In case of inherited disorders, it impacts multiple members of the families as well as could be affecting an entire community of endogamous populations. A subgroup of this disease exhibits a phenomenon of anticipation wherein the first generation has the disease when the individual is in 80's. In the second generation it is much more severe and the onset it much earlier and the individuals could be wheelchair bound well before their teens. There is extensive clinical and genetic heterogeneity wherein the disease could be (a) caused by mutations in over 200 genes (b) extensive genetic heterogeneity in ataxia both within and between populations and if the same gene is affected the mutations could be different (c) Mutations in different genes also share overlapping clinical symptoms therefore it becomes difficult for a clinician to recommend a genetic testing. This poses a dilemma for the clinicians in defining the phenotypic sub-group and consequently, there is a reduced diagnostic yield. In a research spanning 20 years we have been providing diagnosis for ataxia in India through an ataxia clinic in AIIMS as well as are catering to genetic diagnosis of the same referred from different parts of the country. This has been built up through an iterative process of clinical diagnosis, genetic testing and re-evaluation through correlation of genetic testing with clinical profiles. Through testing of over 5000 families we have been able to device a clinic-genetic algorithm for cost effective screening of ataxia. In some cases, through initial observations in over ~10-20 families the clinical diagnosis has become very accurate and the genetic tests are confirmatory. We now know that 50% of the cases can be resolved by 6-8 tests. Using population polymorphism scanning we dissected out origins and regions behind genetic disease, this helped us in the understanding endogamous populations, their ancestry and associated risk factors in Indian population. We have now also been able to identify 3-4 communities where more than 200 families are impacted by a single mutation and the community has approached for preventive screening. To identify novel gene or unidentified mutation in uncharacterized cases, we have used next generation sequencing technologies and resolved nearly 50% of the unresolved cases. For translation perspectives we have also created a bio-repository of clinical samples and patient derived cell line which is not only helpful in validation of novel mutation but led us advancement in understanding disease mechanisms and progress towards effective disease management. An overview of the challenges and promising paradigm from diagnostics to therapeutics interventions of CAs would be provided.

Lysosomal Storage Disorders in Indian Children with Neuro – Regression

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Neuro-regression in childhood could either be genetic with neurometabolic origin or non-genetic causes such as infections

and toxins. It has been observed that more than two third of the diagnosed cases of progressive neurological decline are due to metabolic disorders. Approximately 4.5% of the cases have mitochondrial disease and several are found to have basic metabolic abnormalities like vitamin B12 deficiency and thyroid disorders. Lysosomal storage disorders (LSDs) are the heritable group of nearly 40 heterogeneous disorders occurring due to genetic defect in one or more specific lysosomal enzymes, activator protein or membrane protein resulting in deficient enzyme activity. There is very little information available regarding the role of LSDs in neuroregression, except for few studies demonstrating neurological deterioration as the most commonly occurring pathophysiology of LSDs in around one-third of the cases. Though, individually these disorders are rare (incidence 1:1,00,000), collectively they occur with the frequency of approximately 1:7000-8000 live births. Availability of prenatal diagnostic facilities, newborn screening and the possibilities of early therapeutic approaches has increased awareness among medical fraternity for different LSDs. Therefore, we studied the frequency of various LSDs as the cause of neuro-regression in children from India. In total 432 children (age range: 3 months to 18 years) having regression in a learned skill were selected from 1453 patients referred for diagnostic workup of various LSDs. Plasma chitotriosidase, quantitative and qualitative glycosaminoglycans, and mucolipidosis-II/II screening followed by confirmatory enzyme study using specific substrate was carried out; Niemann-Pick disease Type-C was studied by fillipin stain method on skin fibroblasts.Total 309 children (71.5%) were diagnosed with different lysosomal storage disorders as the underlying cause of neuroregression. Plasma chitotriosidase was raised in 82 of 135; 64 (78%) of these had various LSDs. 69 out of 90 cases showed high excretion of glycoaminoglycans, and 67 (97.1%) of these were confirmed to have enzyme deficiency for various mucoplysaccharide disorders. While 3/90 children with positive I-cell screening had confirmed mucolipidosis-II/III disease. Among all, glycolipid storage disorders were the most common (50.2%) followed by mucopolysaccharidosis (21.7%) and sulphatide degradation defect (17.5%). Neuronal ceroid lipofucinosis-1 & 2 (7.4%), mucolipidosis-II/III (1%), Sialic acid storage disorder (1%), Niemann-Pick disease type-C (1%) and Fucosidosis (0.3%) were observed with less frequency. Most common phenotypes in all subjects were cherry red spot (18.5%), hepatosplenomegaly (17.9%), coarse facies (15%), seizures (13.1%) and skeletal abnormalities (12.14%). Lysosomal storage disorders are considered to be one of the common causes in children with regression in learned skill, dysmorphic features and cherry red spot. Among these, glycolipid storage disorders are the most common, followed by mucopolysaccharidosis.

Excavating Pragmatic Drug Targets to Suppress the Pathogenesis of Poly (Q) Mediated Neurodegenerative Disorders

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Polyglutamine or poly (Q) disorders such as Huntington's disease, spinocerebellar ataxia, spinal and bulbar muscular atrophy etc. are dominantly inherited irreversible devastating human illnesses which are characterised by progressive loss of selective neurons in adult brain. The hallmark of these diseases is the unusual increase of poly (Q) repeats within the coding region of the target gene which leads to misfolding of the encoded protein and aggregate formation. Such neurotoxic protein aggregates are known as inclusion bodies and triggers disease pathogenesis. Owing to various limitations attached with human genetics and due to rare availability of brain tissues, model systems such as *Drosophila melanogaster* has been widely used to investigate the mechanistic in-depths of disease pathogenesis, and to develop effective treatment strategies. The study was directed to identify and characterize novel genetic suppressor (s) of human poly (Q) disorders which could be utilized as effective drug targets to mitigate the pathogenesis of poly (Q) disorders. Human poly (Q) disorders were recapitulated by achieving targeted expression of desired human mutant protein with 78 or more poly (Q) repeats which causes manifestation of neurodegenerative phenotypes in *Drosophila*. Comprehensive genetic screening was performed to identify the potential genetic suppressor (s). Subsequently, various classical and contemporary genetic, cellular, molecular and biochemical strategies were adopted to investigate and decipher the mechanistic details of the rescue event. Following an extensive genetic screening and subsequent analysis, we have demonstrated for the first time that tissue specific over-expression of *Drosophila* Myc (dMyc) or human c-myc proto-oncogene could dominantly suppress the poly (Q) induced neurodegeneration in *Drosophila*. Intriguingly, up regulation of dMyc/cmyc significantly restricts poly (Q) mediated neural deaths and also causes notable reduction in accumulation of toxic inclusion bodies. Our subsequent analysis establishes that dMyc mediated suppression of poly (Q) toxicity is primarily achieved by alleviating the cellular level of CREB-binding protein (CBP). Improved level of CBP in turn restores the status histone acetylation, resulting in reinstatement of transcriptional machinery which was otherwise abbreviated due to poly (Q) disease conditions. In addition to above, we have also demonstrated an active involvement of Insulin signalling pathway (InR) in pathogenesis of human poly (Q) disorders. Our recent findings establish that insulin signalling pathway as a potent modulator of human poly (Q) disorders. We have identified novel genetic suppressors of poly (Q) disorders and also established the cellular and

molecular of mechanism of the rescue event. The identified genetic suppressors could be potentially utilized as pragmatic drug targets and to develop innovative strategies to combat the devastating human neurodegenerative poly (Q) disorders.

Recent Advancements in the Understanding of Neurodegenerative Diseases Based on Large Scale GWAS, Exome and Whole Genome Studies

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The recent advances in the understanding of the genetic architecture of neurodegenerative disorders has been fuelled by large scale genome wide association studies (GWAS) and coming together of large consortia from across the world to meta-analyze these large scale GWAS. The coming together of IGAP, ADGC and other consortia to discover 21 genes for Alzheimer's disease (AD) is a very good example (Lambert et al, Nat Genet 2013). Till recent years the major contribution was from studies on samples of European ancestry, but recent addition of non-European samples has given new insights by discovery of new gens and helped further refine the GWAS signals bringing us closer to the causal variants (Chauhan et al, Nat Genet 2018). In case of some fatal disorders like stroke there has also been contribution from large longitudinal population-based studies in discovering new genes, which was not possible by the usual hospital-based case-control studies because the subjects could not reach the hospital in time (Chauhan et al, Lancet Neurol 2016). Many next generation sequencing (NGS) studies employing whole genome and exome sequencing are also under way to discover the low frequency genetic variants and regions missed by GWAS. Many large-scale studies from India are also underway to fill the missing gap from the Asian population in the understanding of neurodegenerative disease like AD and stroke. This presentation will focus on the large scale GWAS and sequencing studies that have been conducted so far along with an introduction to the studies being conducted in India.

Neurological Disorders due to Gene Based Channelopathies

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Ion Channels are present in the cell membrane and they are proteinaceous in nature. There are two types of ion channels voltage gated and ligand gated. The ion channels are present in all cells and it is especially important in nerve cell function. The ions involved are sodium, potassium and chloride. Mutations in the ion channels may lead to neurological disorders. Some of the disorders which are gene based seen in the practice are, Dravet syndrome mutation at SCNA1, other epileptic encephalopathies due to SCN2A mutation, AD nocturnal frontal lobe epilepsy due to CHRNA4 and other gene mutations, episodic ataxia due to KCNA1 and CACNA1A gene mutation, familial hemiplegic migraine due to CACNA1A, ATP1A2 and SCN1A mutation, familial startle disease due to mutation at GLRA1, sodium channel mutations leading to painful neuropathy syndromes, mutations in acetylcholine receptor result in congenital myasthenia, myotonia congenita due to CLCN1 mutation, hypokalemic periodic paralysis due to CACNA1S mutation, hyperkalemic periodic paralysis due to SCN4A mutation and thyrotoxic periodic paralysis due to specific gene mutation. There are many more neurological disorders due to gene based channelopathies. Ongoing work in the future will give better management of these disorders. In this presentation clinical case of periodic paralysis, congenital myasthenia and myotonia congenita will also be discussed.

A Search for Genomic Determinants in a Unique Familial Epilepsy Syndrome – An Experience

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My talk will touch on my experience and travel in unravelling some interesting clinical and demographic details of ADCME. Autosomal dominant cortical tremor, myoclonus, and epilepsy (ADCME) or familial adult onset myoclonic epilepsy (FAME)/benign adult familial myoclonic epilepsy (BAFME) is a non-progressive disorder with an age of onset of 12–50 years characterized by distal action and postural tremors that resemble essential tremors, exacerbated by fatigue and emotional stress; stimulus sensitive myoclonus that is arrhythmic and segmental, predominantly seen in the upper limb and aggravated by photic stimuli, fatigue, emotional stress, and sleep deprivation and seizures that were mostly generalized tonic–clonic in type, with few patients having complex partial seizures. The electroencephalography (EEG) pattern in these patients was either generalized spike and wave discharges with photosensitivity or focal EEG abnormalities. The somato-sensory evoked potential (SSEP) study is reported to show giant cortical potentials suggesting cortical origin for the myoclonus, whereas no significant neuroimaging abnormality has been reported. These patients had significant clinical improvement with antiepileptic drugs (AEDs)—namely valproate and clonazepam. ADCME/FAME has an autosomal

dominant inheritance with a high degree of penetrance and has been reported in families worldwide with mutations in different genetic loci-Japanese families (8q23.3-q24.1), Italian families (2p11.1-q12.2), 1 French family (5p15.3.1-p15.1), and a Thai family (3q26.32-q28). De Fusco et al. provided evidence of an AR alpha 2B involvement in ADCME. This entity is still not recognized as an independent entity under the ILAE. This entity has been reported in one family in northern India recently, whose genetic defect is not known. We have made possibly the world's largest report of ADCME in south Indian communities reported in 446 patients belonging to 81 families domiciled in southern districts of Tamilnadu belonging to a specific community. The genomic studies in these patients is being ongoing and is in advanced stages with a very challenging travel of more than 2 years. We acknowledge the CSIR-IGIB, New Delhi for the scientific and technical support.

Dissecting the Genetics of Intracranial Aneurysm in Indian Population

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Intracranial aneurysm is a common condition with around 6% of the general population might carry an unruptured aneurysm. It is often asymptomatic until the time of rupture and lack of precise phenotype, biochemical or even imaging based early diagnostic markers puts patients into risk of rupture. Aneurysmal rupture is a potentially lethal event with a mortality rate as high as 50 percent of which 12% die before any medical attention could reach them. From remaining 50% of the patients who survive the initial rupture can undergo severe neurological deficits. The incidence of cerebral aneurysm has strong ethnic bias. Incidence of SAH from aneurysmal rupture varies from 0.75% to 10.3% and has shown a sharp increase in the past decade. Racial or ethnic differences in allele frequencies among populations were observed in several genetic association studies. Candidate-gene studies based on individual hypothesis and linkage studies based on positional approach have laid a strong foundation for IA genetics. The technological advancement of high throughput genotyping has provided a powerful tool to examine the genetic basis of these diseases through Genome-Wide Association Studies (GWAS). GWAS in intracranial aneurysm have identified several new markers. These candidate genes, linkage regions and GWAS hits have yielded conflicting results in different ethnicities, which further increased the complexity of IA genetics. These contradictions may be due to genetic heterogeneity, or possibly even due to varying phenotypes. Therefore, we were interested to understand the complexity, ethnicity and asymptomatic nature of the disease by initiating a genetic association study based on candidate gene approach and a replication of GWAS studies in Indian population. Details of this study will be presented during the meeting.

Genetics: Where it has Impacted Most in Routine Neurological Practice Today

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As the number of commercial genetic tests increases and clinical whole-genome sequencing has become a reality, the interpretation of these tests has become more challenging for the practicing neurologist since these tests have the power to detect new genetic variants. Such variants of 'unknown significance' are a big puzzle to the practitioner and explaining these findings to the patient and family needs clear guidelines. While trying to interpret the results of genetic tests, attention must be paid to some of the variants of unknown significance which affect the interpretation. As more susceptibility and other genes are identified for common disorders, genetic counselling will become increasingly more common in the care of individuals with neurological conditions and will play an increasing role in the multidisciplinary approach to predicting, diagnosing and managing neurological disease. Keeping this in mind, our healthcare sector must start training professional genetic counsellors to evaluate genetic risk, communicate complex medical information, provide decision support, and order and interpret genetic test results. Counsellors should also be trained to work with neurologists to support patients' psychological adjustment to the integration of new information. Neurologists should establish a relationship with the genetic counsellors in their area. The pro-band or initial patient is not always the best person for genetic testing and the best candidate for testing must be identified by a family history, with consideration on whom testing will provide the least potential anxiety. A genetic Information Non-discrimination Act, like the US Act, must be formulated by our health departments to protect asymptomatic individuals from employment and health insurance discrimination based on genetic information, with ample modifications to suit cultural practices and the legal framework and laws of our country. Utilizing genetic information appropriately, such as interpreting genetic test results or recognizing at-risk individuals or ensuring the genetic safety of pharmaceutical agents must be increasingly emphasized in the practice of medicine at all levels to protect healthcare practitioners from costly litigations.

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Oral Presentations

Utility of Next Generation Sequencing in the Diagnosis of Infantile Epileptic Encephalopathy: Case Reports

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Background: The International League against Epilepsy defines epileptic encephalopathies as conditions in which the epileptiform abnormalities are believed to contribute to progressive disturbance in cerebral function. Early infantile epileptic encephalopathy-6 (IEE) also known as Dravet syndrome (OMIM#607208), generalized epilepsy with febrile seizures plus type 2 and familial febrile seizures-3A. Analysis of gene mutations in IEE will help in confirmation of the diagnosis which will translate to appropriate management in contrast to generalized treatment.

Purpose: To compare the spectrum of mutations in patients with epileptic encephalopathy. We present the mutations observed in two cases of infantile epileptic encephalopathy. Case 1: A 5-year-old male baby, born of a non-consanguineous marriage, presented with clinical indications of recurrent seizures with fever and learning or behavioural issues. He is suspected to be affected with Dravet syndrome. Case 2: 1 year old female baby born of a non-consanguineous marriage, presented with clinical indications of seizures since 5th month of life, refractory seizures, multiple episodes of seizures per day, myoclonic jerks, regression of milestones, unable to crawl and elevated levels of glutamine and alanine, suspected to be affected with pyridoxine-dependent seizures or West syndrome. Both the samples has been evaluated for pathogenic gene variations.

Methods: Targeted gene sequencing: selective capture and sequencing of the protein coding regions of the genome/genes is performed. DNA extracted from blood was used to perform targeted gene capture using a custom capture kit. The libraries were sequenced to mean >80-100X coverage on Illumina sequencing platform. The sequences obtained are aligned to human reference genome (GRCh37/hg19) and variants relevant to the clinical indication were analysed.

Results: Case 1: A heterozygous 5′ splice variation in intron 24 of the *SCN1A* gene (chr2:166852522C>A) that affects the invariant GT donor splice site downstream of exon 24 (c.4581+1G>T; ENST00000303395) was detected. Case 2: A heterozygous missense variation in exon 9 of the CDKL5 gene (chrX:18602452G>A) that results in the amino acid substitution of Glutamine for Arginine at codon 178 was detected. Early infantile epileptic encephalopathy-2 also known as X-linked dominant infantile spasm syndrome-2 A heterozygous missense variation in exon 26 of the *SCN1A* gene (chr2:166848930T>C) that results in the amino acid substitution of Valine for Methionine at codon 1619 was detected.

Conclusion: Sequencing this variant in the parents and the other affected and unaffected members of the family is recommended to identify the inheritance of the condition. Due to its clear inheritance pattern genetic testing impacts the family in terms of reproductive decisions. Targeted sequencing represents a cost-effective approach to detect variants present in multiple/large genes in an individual.

Computational Analysis of miRNA – Based Regulation in Alzheimer's Disease

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Background: Alzheimer's disease is an irreversible and progressive neurological disorder that destroys the cognitive skills in brain. In the era of post genomics, miRNA have been identified to play a vital role in the signalling pathways implicated in various disorders.

Purpose: To establish miRNA as a diagnostic and therapeutic biomarker for Alzheimer's disease and thereby clarify and develop novel insights into the pathophysiology of Alzheimer's disease.

Methods: Initially, the significant genes associated with Alzheimer's were retrieved from GWAS catalog and the significant miRNAs along with transcription factors were retrieved from miRTarBase and RegNetwork. Then, a regulatory network of Gene-miRNA-Transcription Factors was constructed using Cytoscape and the most regulated genes and their interconnected regulators were analysed using Cytohubba. Seed pairing of studies of gene-miRNA were analysed using miRmap and thus the most significant regulatory networks were obtained, and a further downstream pipeline was proposed. Enrichment analysis followed by functional annotation of the pathways associated with regulation was used to add to the existing knowledge that currently prevails in the pathophysiology of Alzheimer's disease.

Results: Text mining of Alzheimer's in GWAS resulted in 5 significant genes (APOE, BIN1, ABCA7, MS4A6A and

CD33) in all ethnicity. The 5 significant genes contain sites for 8miRNAs (hsa-miR-335-5p, hsa-miR-335-5p, hsa-miR-587,hsa-miR-656,has-miR-335-5phsa-miR-199a-5p,hsa-miR-1908-5p and hsa-miR-199a-3p) with the association of 27 transcription factors (AR, CTCF, ESR1, JUN, NFKB1, NFYA, NFYB, NFYC, PPARG, SP1, STAT1, STAT2, USF1, USF2, TFAP2A, ABL1, BIN1, CREB1, CUX1, MYC, TBP, TP73,EBF1, MAX, SPI1, YY1 and GABPA). Among various regulatory networks, it was identified that APOE gene contains more interconnections with the regulators (hsa-miR-199a-5p,hsa-miR-1908-5p, hsa-miR-199a-3p, AR, CTCF, ESR1, JUN, NFKB1, NFYA, NFYB, NFYC, PPARG, SP1, STAT1, STAT2, USF1, USF2,TFAP2A). Seed pairing of gene-miRNA, resulted in the compatibility of APOE and hsa-miR-1908-5p. Enrichment analysis of genes resulted in the association of ABC transporters pathway.

Conclusion: Based on the analysis of text mining, graph theory and statistical measures, the probable regulatory networks that prevails the pathophysiology of Alzheimer's disease were APOE-hsa-miR-1908-5p- AR, CTCF, ESR1, JUN, NFKB1, NFYA, NFYB, NFYC, PPARG, SP1, STAT1, STAT2, USF1, USF2,TFAP2A. Further research work in miRNA-based biomarker identification, involves the reconstruction and simulation of ABC transporters pathway with hsa-miR-1908-5p and the associated transcription factors to identify the implication of miRNA as a biomarker to treat Alzheimer's disease.

Genetic Testing in Parkinson's Disease Revealed Gender Difference in Expression Patterns of Transcription Factors Associated with Dopamine Neurons

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Background: Parkinson's disease (PD) is the most common age-related neurodegenerative disease. Recently in Deccan Chronicle, it has been published that in India the incidence is bound to double in ten years. Conventional imaging techniques such as MRI and CT scan are usually unremarkable or may show age specific changes in Parkinson's disease. Doctors use medical history and a neurological examination to diagnose it, but 70% of nigral neurons are lost when symptoms appear. Late diagnosis hampers clinical development of new disease-modifying therapies; only alleviating symptoms is possible at present. For this reason, great interest in developing peripheral biomarker for PD has increased.

Purpose: Several studies demonstrated that transcription factors (TFs) like Nurr1, Foxa1, En1/2 and Lmx1a/b are involved in regulation of neuronal development and maintenance of nigrostriatal system function may be risk factors for PD. However, there is no evidence in Indian PD patients. Hence, in the current study the expression of Transcription factors (TF's) (Nurr1, Foxa1, En1/2 and Lmx1a/b) were profiled in peripheral blood lymphocytes (PBL) in a small Chennai population of India.

Methods: In the present study a total of 40 subjects, 12 male PD patients aged 65.85 ± 1.19, 8 female PD patients aged 65.7 ± 1.202, and 20 healthy controls (HC) matched by gender, age, and origin were included. Transcription factors expression in PBL was measured by Real-Time PCR.

Results: In the 20 PD cases TF's expression was significantly reduced in both male and female PD However, Nurr1 (57.631% reduced in males; 28.93% in females), Foxa1 (64.42% in males; 55.76% in females), Lmx1a (39.1% reduced in males; 18.13% in females), Lmx1b (56.41% reduced in males; 31.50% in females), En1-a (41.4% reduced in males; 46.9% in females), En-1b (41.6% reduced in males; 19.4% in females), En-2a (40.7% reduced in males; 31.58% in females), En-2b (49.2% reduced in males; 34.41% in females) mRNA expression did differ greatly between male and female PD patients.

Conclusion: These findings suggest further investigations of more patients that could potentially reveal peripheral molecular marker for accurate early diagnosis of PD and different significance to the respective genders.

Establishment of Induced Pluripotent Stem Cell Lines from an Attention Deficit Hyperactivity Disorder (ADHD) Individual and his Sibling Control

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Background: ADHD is a neurodevelopmental disorder characterized by a lack of attention, hyperactivity and impulsivity and functional abnormalities in the brain. It affects 4-12% in school-age children and impacts the social life of the affected individuals and their families, causing a social and financial burden on the society. Although it is hereditary and many genes such as SLC6A3, DRD4, DRD5, SLC6A4, HTR1B, SNAP-25, LPHN3 and NOS1 have been identified to be associated with the disorder, environmental factors like prenatal stress, abuse, violence, poverty in early life, smoking and

consumption of alcohol and drugs during pregnancy have been shown to increase a person's risk for ADHD.

Purpose: The pathophysiology of ADHD is poorly understood due to the lack of valid cellular models that faithfully represent the clinical features of ADHD, and limited availability of live brain tissues to expand for long-term mechanistic studies. Due to its complexity and huge variation between individuals it has become difficult to study the underlying mechanism behind its manifestation in humans. Although several animal models have been used to understand the underlying molecular mechanisms of ADHD, animal models in general fail to recapitulate the complexity of ADHD as a uniquely human condition. We aim to develop a collection of induced pluripotent stem cell lines that are to be differentiated into different brain cell types to understand the pathophysiology of ADHD.

Methods: Peripheral blood mononuclear cells (PBMCs) were collected from an affected child and his unaffected sibling control. Diagnoses were made using the Diagnostic and Statistical Manual of Mental Disorders V (DSM-V) criteria, Revised Conner's' parent rating scale and the Child Behaviour Checklist (CBCL). PBMCs were reprogrammed into induced pluripotent stem cells (iPSCs) using the CytoTune-iPS 2.0 Sendai Reprogramming Kit. Reverse transcription-polymerase chain reaction (RT-PCR) was performed to check for the absence of viral residue and G-banding karyotyping for chromosomal abnormality. Pluripotency was confirmed with the expression of biomarkers using immunocytochemistry analysis and alkaline phosphatase staining. Further work to validate the differentiation capacity of the iPSC is underway.

Results: Karyotyping revealed that all the chromosomes carried normal diploid phenotype. RT-PCR confirmed the absence of any viral residue. Distinct fluorescence was observed for TRA-1-60 as it is a membrane protein in the cell surface as opposed to DAPI that stains the nucleus while overlapping fluorescence was observed with Nanog that functions in the nucleus and DAPI. Brown-black precipitation formation of the cells indicates the high level of expression of alkaline phosphatase enzymes in iPSCs.

Conclusion: Thus, our collection of ADHD-iPSC lines serves as an invaluable tool to study the biochemical, molecular and electrophysiological properties of various brain cell types underlying ADHD pathology. To our knowledge this is the first study on the iPSC generation from peripheral blood samples.

Systems Approach in Neurodegenerative Conditions – Neuroinflammation

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Background: Neurodegenerative conditions are related to practical loss of brain cells resulting in deterioration of cognitive abilities with advancing age in world wide population. Many of these conditions occur due to genetic and non genetic factors such as presence of foreign particles implying its role in Alzheimer, Parkinson, Huntington, CNS injury and so on.

Purpose: Loss of neurons due to homeostasis imbalance triggers pathogenic mechanism which leads to chronic progression of the disease. Neurotoxicity in brain leads to neuroinflammatory activity dysregulation misguiding neuroprotective signals resulting in further cellular damage and loss of neuronal functions. Hence,understanding the neuroinflammatory pathways role in neurodegeneration conditions through top-down systems biology approach has been our thrust area of research.

Methods: Our analysis of expresssion data from parkinson disorder using R packages to find differentially expressed genes based on log FC(fold change) with unadjusted P-value. The data mining is done from data resources –Gene Expression Omnibus and Allen Brain Atlas. The diseased genes were screened from normal expressing genes to identify signals influencing inflammatory activity by comparitive analysis. The network analysis plugins were used to screen key genes from pathways, signaling neuroinflammation leading to chronic degradation of neurons.

Results: The beta catenin phosphorylation pathway was analysed further in microglial cells to know its role in parkinson pathology. The major histocompatibility complex (MHC) were identified and their frequency were studied in different population. Identified targets from neurodegeneration conditions were studied in biomarker identification, therapeutic and drug repurposing approach.

Conclusion: In neurodegeneration conditions there is a difference between practice of public health and that of clinical neurology. In public health the entire communities are looked after their health concerns whereas it's vice versa in clinical neurology. These two approach are seen as opposite of two extreme ends for neurological condition treatment so my work will be aiming to apply to both of these approaches so the community and the individual person can be benefitted.

A Chemically Induced Zebrafish Model for Huntington's Disease like Phenotype

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Background: Ever since its discovery, Succinate Dehydrogenase (SDH) (EC 1.3.5.1) also known as complex II, with four subunits, has been extensively studied for its dual

functionality in Krebs cycle and oxidative phosphorylation. Loss of activity in any of the complex II subunits leads to a spectrum of disorders from cancer to neurodegenerative diseases. There are many chemicals which could act as potential inhibitors for complex II, and 3-Nitropropionic acid (3NPA) was characterized to be an efficient complex II inhibitor inducing Huntington's disease (HD) symptoms in mouse model system. 3NPA was observed to cause degeneration of neurons in the basal ganglia of the treated mice. 3NPA has been reported to induce oxidative stress leading to neurobehavioral and biochemical changes that mimic the human HD pathological conditions.

Purpose: We intend to generate chemically induced zebrafish model system to characterize the non-canonical functions of complex II and its role in HD phenotype.

Methods: Homology modelling was performed to predict the 3D structure of zebrafish SDH subunits. Subsequently we performed molecular docking to analyse the interactions between 3NPA and complex II. We treated wild type zebrafish with 3NPA and performed various behavioural and biochemical assays to score for HD like phenotype.

Results: Homology modelling and molecular docking analysis showed the interactions between 3NPA and complex II. Behavioural differences were observed between the control and the 3NPA treated zebrafish. We also observed significant decrease in the complex II activity in the 3NPA treated zebrafish sample. Various oxidative stress assays demonstrate HD like phenotype in the 3NPA treated zebrafish.

Conclusion: In this study, we have generated a chemically induced vertebrate HD model, which can be used in potential drug screenings.

Decoding the Mechanisms of the Neurotransmitter Dopamine Catabolism by Monoamine Oxidase B

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Background: Dopamine is a neurotransmitter which plays a crucial role in motor control, motivation, mood, memory, reward, etc. Altered levels of dopamine are associated with neurological diseases like Parkinson's disease (PD), Alzheimer's disease (AD), attention-deficit hyperactivity disorder, and schizophrenia.

Purpose: PD is characterized by loss of dopaminergic neurons in the substantia nigra and increased levels of the dopamine degrading enzyme, monoamine oxidase B (MAO-B). Levodopa and monoamine oxidase B (MAO-B) inhibitors are routinely administered to treat motor symptoms associated with PD. However, the regulatory mechanisms of MAO-B gene expression remain incompletely understood. In this study, we set out with an aim to delineate the molecular mechanisms involved in MAO-B gene regulation.

Methods: We have utilized various bioinformatic tools, reporter assays, Western blotting, and electrophoretic mobility shift assays (EMSAs), chromatin immunoprecipitation (ChIP) assays, to systematically delineate the roles of these factors involved in MAO-B gene regulation.

Results: Generation of varying lengths of promoter-reporter constructs followed by transfection into various cell lines led to identification of core promoter region (viz. -144 to +25 bp). Stringent in silico analysis of the core promoter revealed putative binding sites for Sp1/Egr1/CREB. Similarly, in silico analysis of MAO-B 3'-UTR predicted binding sites for miR-1224/miR-300. Over-expression/down-regulation of Sp1/Egr1/CREB and miR1224/miR-300 resulted in increase/decrease in the MAO-B reporter activity and endogenous protein levels. Electrophoretic mobility shift assays (EMSA) using labelled/unlabelled wild-type/mutant MAO-B promoter oligonucleotides displayed formation of specific complexes; the identities of these complexes was established using specific antibodies. Consistently, the in vivo interaction of Sp1/Egr1/CREB with the MAO-B promoter was confirmed by chromatin immunoprecipitation (ChIP) assays. Furthermore, dopamine dose-dependently enhanced MAO-B promoter-reporter activity and endogenous protein levels via (i) enhancement of CREB, (ii) reduction of miR-1224 and miR-300 levels. 8-Br-cAMP and forskolin augmented the promoter-reporter activities, whereas PKI (PKA inhibitor) diminished the promoter-reporter activity suggesting involvement of cAMP-PKA axis in regulating MAO-B expression.

Conclusion: Taken together, this study elucidates the role of Sp1/Egr1/CREB, miR1224/miR-300, and dopamine in governing MAO-B gene expression which might have implications for pathological conditions involving dysregulated catecholamine homeostasis.

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3rd Madras Medical Mission Genetics Meeting 2018
Symposium on 'Advances in Genetic Diagnosis of Neurological Disorders'

Poster Presentations

A Systems Perspective to the Understanding of Migraine – Epilepsy Genetics

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Background: Migraine is a common neurological disorder characterised by recurrent headaches that are moderate to severe. Several factors such as allergies, aura and light may lead to migraine. Hormonal, emotional or physiological changes may also cause migraine. But exact cause of migraine is still unknown. CACNA1A, ATP1A2, SCN1A are three ion channel genes that involved in migraine. Mutations in ATP1AT2 that encodes 2 subunit of Na+ /K+ are associated familial hemiplegic migraine. Epilepsy is a group of neurological disorders characterized by epileptic seizures. These seizures can vary from brief and nearly undetectable to long periods of vigorous shaking. The cause of most causes of epilepsy is unknown. Seizures are controllable with medication in about 70% of cases.

Purpose: The study is to be our first step in understanding the genetic regulatory interactions between migraine and Epilepsy as comorbidities by identifying the Genes and genetic interactions through the medium of microRNAs. We propose to study the pathologic – genetic basis between these comorbidities from network biology perspective.

Methods: We used disease gene association databases like DisGeNEt were used to mine for established links between genes. Implicated microRNAs and MicroRNA target prediction tools like Targetscan were used to obtain the gene – miRNA networks. Network biology tools and protocols were then used to understand, analyse the networks and obtain insights about the diseases. Implicated proteins and their classes were also obtained and functionally annotated.

Results: We sought list the genes of epileptic and migraine etiology and also attempted to survey the kind of protein classed that could possibly be coded from these reading frames. A list of the protein classes were obtained from the entirety of the epileptic genes we discovered. These co – morbidities hint the underlying network biology of shared and multifunctional genes and pathways. Of the many genes implicated in the etiology of the many types of epilepsy and migraine, 1643 were unique for epilepsy and 749 were unique for migraine types. 544 genes were understood to be common between the epilepsies and migraines. On a systems level, we also identified cooperatively regulating miRNAs in the miRNA association network. MicroRNAs can bind to one gene and the target sites may overlap to some degree. The individual effect of a miRNA may appear to be small but when they cooperate, the effect can be of significant proportions.

Conclusion: It is well known that migraine and epilepsy are episodic disorders that share many clinical features and underlying pathophysiological mechanisms. A hallmark of episodic disorders is that they often are due to defects in ion channels, or more generally, ion-translocating transmembrane proteins including Na+, K+-ATPase. As episodic disorders, epilepsy and migraine share certain common characteristics and a presumption that the underlying pathohysiology relates to alterations in ion channels or ion transporters.

Association of Amine Oxidase Copper Containing-1(AOC 1) Gene with Migraineurs

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Background: Migraine is a neurovascular disorder associated with biochemical abnormalities of central nervous system. Amine oxidase copper containing 1 (AOC1) gene encodes diamine oxidase (DAO) which acts extracellularly and deaminateshistamine. DAO contains tightly bound copper as one of the cofactors.

Purpose: The aim of this study is to determine the association of AOC1 gene and serum copper levels in migraineurs.

Methods: Serum CU levels were determined using atomic absorption spectroscopy and compared with the normal levels in 100 migraineurs and 100 controls. Variations in exon 4 of AOC1 gene were analysed using PCR-SSCP techniques.

Results: In this study, serum CU levels were found to be significantly lowered in migraineurs and compared with AOC1 gene.

Conclusion: The decreased CU levels and variations in AOC1 gene may reduce the activity of diamine oxidase thereby increasing the risk of migraine.

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Gene Prioritization in Alzheimer's disease and Further Functional Annotation Reveals Connection to Longevity and Natural Atrophy

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Background: The contribution of network biology to the understanding of biology and medicine has been phenomenal. The most significant contributions have been in the understanding of complex disorders including neurodegeneration. It has become a very important player in a systems approach to understanding disease. In this study, we have used this approach to improve our understanding of the complex interactions that occur between genes and proteins in Alzheimer's disease (AD).

Purpose: The combination of genetics, molecular biology and biochemistry has produced remarkable advances in our understanding of the causes and mechanisms of AD, and all of these sciences are required for therapeutic approaches. Genetic and genomic studies have resulted in hundreds of genetic loci in neurodevelopmental disorders and neurodegeneration. With a focus on network perspective, this study reveals how high-throughput molecular, integrative data and network approaches inform disease biology. This provides a framework for interpreting network biology studies and leveraging big genomic data sets in neurobiology.

Methods: Due to the complexity of the disease and the numerous genetic players involved, we are attempting to construct a molecular interaction network of interactions of the genes, proteins and other molecules in Alzheimer's disease [AD]. This global network will then be topologically analysed, as a whole, in order to translate the experimental molecular connections into topological motifs, meaningful, in the identification of genes with proteins and its association types and which would possibly aid in the diagnosis, and therapy of AD. Genome annotation tools like DAVID were used to enrich and cluster the gene set.

Results: With a total of 1443 genes and all their protein counterparts the reconstructed network comprised of an overwhelming 26, 490 nodes and 25045 edges. Some of the genes include the etiologically well-established APP, APOE, CLU, PCDH11X, TOMM40 and MIF590. From the network we identified top 20 interacting genes and the list included – HLA-A, HLA-DRB1, CYTB, ND5, ATP6, ND1, ND2, COX1, COX3, ND4, ND3, COX2, ND6, ND4L, ATXN3, ATP8, TP53, BRCA1, CCR5 and DRD4. These were the topologically and hence functionally most significant genes implicated in Alzheimer's disease. It was understood from annotation that the binding properties in the intracellular proteins, ions and nucleotides play a crucial role in the pathophysiology of the disease.

Conclusion: Many of the genes which play prominent role in cellular response to stimulus also were implicated as the third major player from the results of annotation reminding us further strongly that the disease does a very important environmental component and is thus a 'complex disease'. Stuctural, developmental and proliferation genes as well as those responsible for cell death were also considered to be key in the pathophysiology indicating that longevity genes and the natural atrophy of cells during the process of aging definitely facilitate the disease progression.

A Computational Analysis toward Novel Insights into the Cellular Senescence and the Aging of the Brain as Observed through Disparate Molecular Phenomena across Multiple Genera and the Neurodegenerative Connection

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Background: Aging is a mysterious process. It deduces from changes occurring simultaneously in different tissues due to intrinsic cellular mechanisms or changes in one tissue may be predominant. Comparing the model organisms with humans by building protein-protein and gene-protein interaction networks, here we have compared humans aging with other model organisms based on molecular interactions.

Purpose: The aim of this studies is to identify protein/gene/transcription factor interactions among themselves that affect aging, longevity, senescence in humans, and other model organisms and to yield further insights into the molecular mechanisms of aging and age-associated diseases.

Methods: Numerous databases were used including GenAge and AnAge, and the genera compared included *Mus musculus*, *Drosophila melanogaster*, *Saccharomyces cerevisiae*, and *Caenorhabditis elegans.* Functional annotation, pathway and network analysis were performed using tools including STRING Reactome and Cytoscape. A list of significant pathways was found based the XD-score. Regression analysis was performed. Enrichment Analysis was done in the Cytoscape environment using the JEPETTO plugin. Linear regression analysis and Pearson correlation of XD-scores as obtained with the JEPETTO application versus the corrected p-value of the Fisher exact test. XD values greater than the intercept of the linear model were considered significant.

Results: A list of ageing related genes was obtained from GenAge. There were 304 human, 126 mouse and 170 drosophila genes. Simple parameters for humans provide

with the information like clustering co-efficient which is 0.529 for a total of 302 nodes. The network diameter was 4 which shows that it is a dense network. Each node has an average of 45.689 neighbors, and the characteristic path length is 2.022. Drosophila's BC plot seems more much more widespread than the two other species. In humans, TP53 was the most interactive gene in the network and AKT1 as the second most interacting. In mouse, TRP53 has the highest degree followed by AKT1 and in Drosophila, Drosophila where AKT1 had the highest degree. MYC gene was common between humans and mouse and PTEN was between mouse and Drosophila.

Conclusion: Gerontology must be more than curiosity-driven, even though the use of model organisms is inevitable. Because the human aging process takes decades to develop it is virtually impossible to study it in vivo. Neurotrophin signaling pathway had an XD score of 1.009 and an overlap size of 35/121. The results were overall consistent between mouse and humans. Since the basic blocks of life are common to most known species, common pathways might be involved in aging across phylogeny. It as well could be that the weakest pathway succumbing to senescence is the same in all of these model organisms.

Parkinson's Disease

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Background: Neurodegenerative disorders are the disorders which show the abnormal behaviour and cognitive characters during postnatal life. Parkinson's disease is the second commonest neurodegenerative disorder. Parkinson's disease is a neurological disorder in which there is a gradual loss of brain cells that make and store dopamine. Dopamine is a chemical in the brain, known as neurotransmitter, which sends messages that control movement. As Parkinson's disease progresses, more dopamine neurons in the brain are lost. The primary symptoms of Parkinson's disease are movement related, and include: resting tremor, rigidity and slowness of movement. However, many patients also experience non-movement related symptoms such as cognitive impairment, mood changes, and constipation and blood pressure problems. The causes of Parkinson's disease remain unknown, although researchers believe the disease may be brought on by a combination of environmental and genetic factors.

Purpose: The treatment available for this disease help to reduce some of the symptoms but there is currently no treatment that can slow or stop the disease from progressing over time.

Methods: Risk factors that have been identified include: advancing age, family history, male gender, exposure to toxins such as herbicides and pesticides, head injury. Not all patients will experience every symptom, and the pace at which the disease progresses can vary on an individual basis. There are different treatment approaches like: dopaminergic strategies, anthicholinergics, Surgical therapies, Exercise, etc. In addition to these symptoms, treatments can bring on side effects that patients should be aware of and discuss with their doctor.

Results: Researchers estimate that one million people in the United States, and four to six million people worldwide, are living with Parkinson's. The average age of onset is 60 years old though some are diagnosed at age 40 or even younger.

Conclusion: As our population ages, the number of people with Parkinson's is expected to grow. Much research is ongoing to identify strategies for improving treatment of Parkinson's disease in the future.

A Diagnostic Dilemma in a Suspected Case of Rett Syndrome

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Background: Rett syndrome is a dominant X-linked male-lethal disorder largely caused by mutations in the gene encoding methyl-CpG binding protein 2 (MECP2). Clinical presentations include neurodevelopmental disorder characterized by early-onset intractable seizures, severe developmental delay, intellectual disability, and abnormal electroencephalograms. Affected females show normal development until the age of 6 to 18 months, followed by gradual loss of speech abilities, microcephaly, social impairment, ataxia, and stereotypic hand movements.

Purpose: To describe a case presenting with typical features of Rett syndrome and discuss the implications of the genetic findings for the family.

Methods: A baby born of a consanguineous marriage, presented with clinical indications of developmental regression, seizures, flapping hand movements and hypotonia, typical of Rett syndrome. The patient was evaluated for pathogenic gene variations by next generation sequencing.

Results: No pathogenic or likely pathogenic variants causative of the reported phenotype was observed. However, interestingly a heterozygous missense variation in exon 10 of the *CIC* gene (chr19:42794581C>T) that results in the amino acid substitution of Leucine for Serine at codon 554 (p.Ser554Leu) was detected. Though this was classified as a variant of unknown significance, the *in-silico* prediction of the variant was possibly damaging by PolyPhen-2 (HumDiv),

probably damaging by PolyPhen-2 (HumVar) and damaging by SIFT.

Conclusion: The phenotype for the detected heterozygous missense variation in exon 10 of the *CIC* gene is autosomal dominant mental retardation-45 (OMIM#617600). This disorder is characterized by delayed psychomotor development, intellectual disability, developmental regression, learning difficulties, poor speech, seizures and nonspecific white matter abnormalities seen on brain imaging. Since these features are similar to Rett syndrome, this case report highlights the importance of genetic testing in neurological disorders for the confirmation of a diagnosis. For this family considering that germline mosaicism for the mutation in the parents was a possibility, identification of the pathogenic variation allowed for prenatal diagnosis for the next child.

Neurodegenerative and Neurodevelopmental Disorders from a Systems Perspective

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Background: System biology is the biology that gives the experimental and theoretical information of the behavioural of the biological systems. It describes how all elements of a biological system interact to understand model and to predict aspects of emergent biological process. The study of neurodevelopment and neurodegenerative disorders through system perspective is a challenging concept.

Purpose: Neurodevelopment disorders are the disorders which are characterized by the abnormal behaviours and the inability of the mental functions that deals with logic, during the early postnatal life. Neurodegenerative disorders are the disorders which show the abnormal behaviour and cognitive characters during the postnatal life. In this study, we set our goals to study the common disease mechanisms between the neurodevelopment and neurodegenerative disorders that can be known through an understanding the underlying systems concepts.

Methods: We focussed on fifteen disorders and worked on their disease genes obtained from the databases such as DisGenet and GeneCards. Out study further narrowed down to most widespread disorders in the population such as schizophrenia, Parkinson's and autism. Schizophrenia and Parkinson are neurodegenerative disorders. Autism is a neurodevelopmental disorder. Using the Disease Connect tool, we found link between Parkinson, schizophrenia and autism.

Results: Using systems biology tools and protocols, we reconstructed molecular interaction networks for Parkinson, schizophrenia and autism disorders. Plugins in cytoscape such as MCODE, BINGO and JEPETTO were used to achieve clustering of networks, gene ontology analysis and functional enrichment analysis respectively. Topological ansy systems analyses were performed. Gene network studies found that, response to cocaine, neurotransmitter biosynthetic process and positive regulation of excitatory postsynaptic membrane potential are the common pathways for schizophrenia, Parkinson and autism disorders.

Conclusion: Thus, we understood that the ECM receptor interaction might be a common reason between the parkinson, schizophrenia and autism. We also understand that there certainly exists a complex link between neurodevelopmental and neurodegenerative disorders.

Analysis of the Interactions of 5-Hydroxytryptamine Receptor 2C with Herbal Compounds – A Bioinformatics Study to Support Epilepsy Treatment

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Background: Epilepsy is a disorder of the Central Nervous System (CNS) in which patients exhibit marked vulnerability to recurrent episodes of excessive neuronal activity in the brain known as seizures. Repeated or prolonged seizures are seriously debilitating conditions and may cause additional pathological changes to the CNS. Nearly 1% of the general population suffer from epilepsy, but many fails to receive significant treatment benefit. The cause of most cases of epilepsy is unknown. Some cases occur as the result of brain injury, stroke, and brain tumours. The 5-hydroxytryptamine2C [5-HT2C] receptors otherwise called as serotonin receptors which are known to trigger epileptic seizures.

Purpose: The main purpose of this study is to develop an enhanced understanding of epilepsy mechanisms which in turn help to explore new possibilities for the effective treatment of currently untreatable forms and those forms accompanied by adverse side effects. It is reported that many 5-HT2C receptor ligand compounds show anti-depressive, antipsychotic and anxiolytic properties, and affecting sleep patterns, feeding behaviour and neuroendocrine functions. Several experimental results suggest that the long-term down regulation of 5-HT2C receptors is partially responsible for antidepressant action.

Methods: Membrane proteins and their complexes play crucial roles in many cellular and physiological processes. The 5-hydroxytryptamine2C receptor is one of the 5-HT receptors with a G-protein-coupled intracellular signalling pathway. The three-dimensional structure of 5HT2C would disentangle answers for many fundamental questions and further provide

enhanced understanding of function, therefore, an attempt is made through bioinformatics approaches to explore the structure using homology-based threading approach. Further molecular docking and molecular dynamics simulations have also been carried out.

Results: More than 800 herbal compounds were identified from several medicinal plants and their interactions were analysed at the molecular and atomic level through modelling, docking and molecular dynamics simulations. Compounds from *Curcuma longa* and *Allium sativam* showed high docking scores (60.2, 66.7) and good number of interactions with the receptor.

Conclusion: Further analysis of the molecular dynamics trajectory reveals that several analogues of curcumin shown up promising results and the findings reported may assist in designing potential inhibitors as well as therapeutic agents and open new avenues for control and treatment of epilepsy.

Novel Formulations in the Treatment of Alzheimer's Disease

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Background: One billion of world population suffer from neurological disorders degenerating health systems. Alzheimer's disease (AD) is currently ranked as the sixth leading cause of death, an irreversible, progressive brain disorder that debilitates gifted cognitive function, language skills and behavioural abilities jeopardizing life. The current available drugs for AD, donepezil, galantamine, rivastigmine and memantine. There have been controversies, restricting their use, hence there is a need for development of novel formulations for AD treatment.

Method: Collecting and referring to the associated literature in recent 10 years, via searching medical subject health terms "Alzheimer's disease; nanotechnology; nanoparticle; formulations, targeted drug delivery strategies, amyloid β peptide; tau protein; autophagy".

Results: In addition to conventional oral formulations for administration of the drugs donepezil, galantamine, rivastigmine and memantine, novel drug-delivery strategies leading to simplified dosage regimes and new pharmacological tools for development of extended release, orally disintegrating or sublingual formulations, intranasal or short- and long-acting intramuscular, transdermal forms, and nanotechnology-based delivery systems plays an exigent role in the treatment of AD.

Conclusion: Formulations with efficacious dosage and delivery is promising in the treatment of AD and improving the quality of patient's life.

The Polygenic Basis and Mitochondrial Genetic Cornerstones in the Suicidal Connections of the Bipolar Disorder

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Background: Bipolar disorder is one of the two major psychotic disorders together with schizophrenia and causes psychosocial disturbances. Metabolic dysfunction and energy supply-and-demand mismatch have been implicated in a variety of neuronal and psychiatric disorders. Exploring the role of mitochondrial dysfunction in the molecular basis of psychiatric disease is a very promising research avenue.

Purpose: The genetics of mitochondrial activity is a cornerstone in understanding disease pathogenesis related to metabolic dysfunction. In line with pathological study, genetic assessment provides an important relationship between biochemistry and clinical correlation in psychiatric disease. The prime purpose of this study was to elicit the interacting molecular species and their inter – networking to generate a molecular interaction map for the bipolar disorder.

Methods: In this study, we probed the mitochondrial genetics – nuclear DNA, mitochondrial DNA, mitochondrial pathways, pseudogenes, nuclear-mitochondrial mismatch, and microRNAs that could contribute to an observable clinical phenotype in manic depression. The databases we used were BD gene and we used the methods implemented therein to determine the core genes involved in the depression, by gene prioritization. We also obtained enriched pathways for those core genes and subsequently carried out pathway-based analysis (PBA). Further, we also enriched the pathways for cross – disorder genes and correlated them with GWAS studies that used the psychiatric behaviour of suicidal tendency.

Results: We used multiple systems biology tools to explore the various aspects of the interactome of this manic disorder; its cellular and metabolic pathways in which mitochondria participate, the mitonuclear crosstalk along with organellar dysfunction in the pathogenesis. Chromosome 2 had multiple loci and multiple SNPs on its multiple genes which had the strongest link to suicidal behaviour in bipolar patients. ACP1 polymorphism (a phosphotyrosine protein phosphatase family Gene) has been associated with suicide attempt in previous studies. The other biomarkers include SAT1, PTEN, MARCKS, and MAPK3.

Conclusion: Through our data mining study, we have understood that the gene list is much longer and thus a gene – gene interaction study as we have done is much needed. We observed disease gene overlap between bipolar, schizophrenia and the major depressive disorder.

A Study on Neuropathic Pain: Causes, Grading System, Mechanism– A Review

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Background: Neuropathic pain, the type of pain condition which is caused by the specific nociceptors and is very different from the regular pain. The pain is highly intolerable to patients as the pain can be evoked by gentle touch, wind, clothes pressure and puts a great challenge to proceed with day to day activities.

Purpose: The treatments for the neuropathic pain reduces the production of neurotransmitters to some extent which be the cause of intolerable pain, but the faster disease progression be the main concern which can be sorted out by earlier prediction.

Methods: The pain distribution in the innervations territories of nerves, roots, fascicles; throughout the body part of central nervous system (CNS); Lesion or the disease which can cause effect on somatosensory system, highly influences the grading system of neuropathic pain. These factors are considered to predict the disease progression.

Results: The efficient grading system is required to differentiate the neuropathic pain condition and to initiate the treatment quickly after the onset of disease. The grading factors give the actual cause, location and the progression of pain.

Conclusion: Understanding the mechanism between the pain conditions and the grading systems are very important to initiate the specific treatments. This review explains about the grading system of neuropathic pain, causes of pain, mechanism and the wide range of drugs involved in the treatment of neuropathic pain.

Genetic Analysis of MTHFR Gene Polymorphisms in Migraineurs

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Background: Migraine is an intricate, polygenic, multifactorial neurovascular disorder, which is characterized by frequent headache episodes. The vascular gene called Methylenetetrahydrofolate reductase (MTHFR) polymorphism has an inconclusive evidence to support its association with migraineurs.

Purpose: This study is aimed to analyse the MTHFR polymorphisms C677T & A1298C among migraineurs.

Methods: Polymerase chain reaction followed by restriction fragment length polymorphism in 200 samples (100 migraineurs and 100 controls) was carried out.

Results: The frequency of the T allele in MTHFR677 was 35.3% in total migraine population as in comparison with the control group which exhibited 27.9% of T allele frequency (OR = 0.71; 95% CI = 0.39 – 1.29). The frequency of the C allele in MTHFR1298 was 33.7% in total migraine population as in comparison with the control group which exhibited 29.35% of T allele frequency (OR = 0.82; 95% CI = 0.45 – 1.50).

Conclusion: Heterogeneity of C677T and A1298C polymorphisms of the MTHFR gene are genetic risk factors for migraineurs, further genotype-phenotype association must be examined closely.

Amyotrophic Lateral Sclerosis – The Research Questions

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Background: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease that affects nerve cells in the brain and the spinal cord. ALS is also known as Lou Gehrig's disease. French neurologist Jean-Martin Charcot discovered the disease in 1869. As ALS progresses; it affects all the voluntary muscles. The person can no longer control their arms, face, and legs. In time, the inability to breathe unsupported can lead to respiratory failure. The types of ALS are – Sporadic – most common, accounts 90-95% of all cases, can affect anyone, anywhere and Familial – also known as FALS (familial amyotrophic lateral sclerosis), inherited, accounts to 5-10% of all cases, in those families there is 50% chance each offspring will inherit this gene mutation and develop the disease. ALS is a difficult disease to diagnose.

Purpose: Lou Gehrig's disease affects 30,000 Americans. The person can no longer control their arms, face, and legs. In time, the inability to breathe unsupported can lead to respiratory failure. Half of all people with ALS will live for 3 years or more after diagnosis, but some live for longer. Prior to the ice bucket challenge, many Americans were not aware of this crippling disease, or of its effects on patients.

Methods: A series of diagnostic tests are performed, often ruling out other diseases that mimic ALS, that a diagnosis can be established. The C9orf72, SOD1, TARDBP, and FUS genes are key to the normal functioning of motor neurons and other cells. Mutations in the C9orf72 gene account for 30 to

40 percent of familial ALS in the United States and Europe. Worldwide, SOD1 gene mutations cause 15 to 20 percent of familial ALS, and TARDBP and FUS gene mutations each account for about 5 percent of cases.

Results: The most prominent goals of ALS research include work toward understanding the cellular mechanisms which lead to the development and subsequent progression of ALS, the discovery of the genetics and risk factors associated with the disease, biomarkers involved, and to develop and improve treatment and management strategies.

Conclusion: Some of the current research avenues include looking into the aetiology of the motor neuron cell death, and the use of stem cells as possible treatments, comparing familial and sporadic ALS cases to identify genes involved in the development of the disease. In this study we have done an extensive survey along these lines the milestones we have crossed and the winding road ahead in research on the Lou Gehrig's disease.

A Review of Systems Approaches Undertaken to Study the Genomic Underpinnings of Obstructive Sleep Apnea

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Background: Obstructive sleep apnea (OSA) is a serious sleep disorder that occurs when a person's breathing is interrupted during sleep. People with untreated sleep apnea stop breathing repeatedly during their sleep, sometimes hundreds of times. OSA is heritable, and there is ample evidence of both direct genetic contributions to OSA susceptibility and indirect contributions mediated through other disease phenotypes like obesity, craniofacial structure, neurological control of upper airway muscles and of sleep and circadian rhythm.

Purpose: Research into the genetics of OSA is an important area and may lead to improved understanding of disease aetiology, pathogenesis, adverse health consequences and new preventive strategies and treatments.

Methods: This paper describes the genetics, genomic and systems level insights we have yet derived on OSA and the questions and complications of that remain. Here investigated two systems biology approaches to detect subnetworks which are likely associated with OSA. An integrated analysis of gene expression, PPI networks, and SNP data from genome-wide association studies have provided novel approaches for combining data from multiple sources to identify candidate pathways for potential validation studies.

Results: It results in diabetes, high blood pressure, migraine, depression and heart failures.

Conclusion: Till now the genetic studies of OSA have lagged behind other chronic diseases but the recent gene discovery efforts have been successful in finding genetic loci contributing to OSA-associated intermediate phenotypes. But still many of the seminal questions relating to the genetic epidemiology of OSA and associated factors remain unanswered.

Understanding the Cotard Delusion – A Review of the Current Research Problems

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Background: Cotard delusion is a rare mental illness in which the affected person holds the delusional belief that they are already dead, do not exist, or have lost their blood or internal organs. Statistical analysis of a hundred-patient cohort indicates that the self-existence is a symptom present in 45% of the cases of Cotard's syndrome, 55% of the patient's present delusions of immortality. In 1880, the neurologist Jules Cotard described the condition as Le délire des négations ("The Delirium of Negation"), a psychiatric syndrome of varied severity. A mild case is characterized by despair and self-loathing, while a severe case is characterized by intense delusions of negation and chronic psychiatric depression. The case of Mademoiselle X describes a woman who denied the existence of part of her body and of her need to eat. In the course of suffering "The Delirium of Negation", Mademoiselle X died of starvation.

Purpose: Research in Cotard delusion is an important area and may lead to improved understanding of disease aetiology, pathogenesis, adverse health consequences and new preventive strategies and treatments.

Methods: This paper describes that the delusion of negation is the central symptom in Cotard's syndrome. The patient afflicted with this mental illness usually denies their own existence, the existence of a certain body part, or the existence of a portion of their body. Cotard's syndrome exists in three stages: (i) Germination stage – the symptoms of psychotic depression and of hypochondria appear (ii) Blooming stage – the full development of the syndrome and the delusions of negation and (iii) Chronic stage – continued, severe delusions along with chronic psychiatric depression.

Results: Typically, patients believe they have lost organs, blood or body parts, or even that they are dead. This relatively rare syndrome exists in patients with depression, schizophrenia and psychotic disorder caused by a general medical condition, and it is often associated with dementia. There are no further diagnostic criteria for Cotard's syndrome within the DSM-5, and identification of the syndrome relies heavily on clinical interpretation.

Conclusion: The successful pharmacological treatments (mono- therapeutic and multi-therapeutic) using antidepressant,

antipsychotic, and moodstabilizing drugs; likewise, with the depressed patient, electroconvulsive therapy (ECT) is more effective than pharmacotherapy Cotard's syndrome resulting from an adverse drug reaction to valacyclovir is attributed to elevated serum concentration of one of valacyclovir's metabolites, 9-carboxymethoxymethylguanine (CMMG). Successful treatment warrants cessation of the drug, valacyclovir. Haemodialysis was associated with timely clearance of CMMG and resolution of symptoms.

The Agenesis of Corpus Callosum– A Review of the Current Research Problem

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Background: Corpus callosum is the major interhemispheric fiber bundle in the brain, and consists of about 200 million axons in humans, that is, approximately 2–3% of all cortical fibers, thus making it the largest fiber tract within the central nervous system.

Purpose: The purpose of this abstract is to focus on the neurological disorder called agenesis of corpus callosum. It describes the causes, symptoms, effects, and treatment for corpus callosum. Formation of the corpus callosum begins as early as 6 weeks of gestation when axons destined to cross the midline can be seen growing medially within the hemispheres. At 11–12 weeks of gestation, the first fibres cross the midline through the massacommissuralis, which is located between the anterior and hippocampal commissures, to form the corpus callosum. Agenesis of the corpus callosum (ACC) is a rare birth defect (congenital disorder) in which there is a complete or partial absence of the corpus callosum. It occurs when the corpus callosum, the band of white matter connecting the two hemispheres in the brain, fails to develop normally, typically during pregnancy.

Methods: Symptoms that may begin early in life are feeding problems and delays in holding the head erect. Sitting, standing and walking may also be delayed. Impairment of mental and physical development, and accumulation of fluid in the skull (hydrocephalus). In some mild cases, symptoms may not appear for many years. Older patients are usually diagnosed during tests for symptoms such as seizures, monotonous or repetitive speech, or headaches. Some patients may have deep-set eyes and a prominent forehead. An abnormally small head (microcephaly), or sometimes an unusually large head (macrocephaly), may be present. Tags of skin in front of the ears, one or more bent fingers, and delayed growth have also been associated with some cases of agenesis of corpus callosum. Agenesis of corpus callosum can be inherited as an autosomal recessive trait or an X-linked dominant trait. This disorder may also be due in part to an infection during pregnancy (intrauterine) leading to abnormal development of the fetal brain. Agenesis of corpus callosum produces symptoms during the first two years of life in approximately ninety percent of those affected.

Results: This condition may also be identified during pregnancy through an ultrasound. Agenesis of corpus callosum can occur in conjunction with spina bifida. Spina bifida is a term meaning open (or non-fused) spine and related services may be of benefit depending upon the range and severity of symptoms.

Conclusion: Treatment is symptomatic and supportive. Anti-seizure medications, special education, physical therapy and related services may be of benefit depending upon the range and severity of symptoms.

Anxiolytic Effects of Tea Polyphenol Nanoparticles in Zebrafish: A Study Using Induced Stress Model

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Background: It is vital for the establishment of effective animal behaviour models for the study and analysis of various aspects of neurobiology and neuropharmacology, and in recent years, zebrafish (*Danio rerio*) have emerged as a robust model for several behaviour studies and drug effect studies (Cerutti et al., 2006). They have proven to be inexpensive, easy to handle and have been used for various aspects of behaviour and cognitive functioning, including systems like that of reward, learning and anxiety. Epigallocatechin-3-gallate, the polyphenol from tea is a potent and well-known dietary antioxidant and has been shown to exhibit effects like those of popular anxiolytic drugs such as chlordiazepoxide in mice as well as cultured hippocampal neurons (Vignes et al., 2006). Anxiety describes a state of elevated fear and restlessness, caused by introduction to an unknown or fearful situation, or can be caused by over-thinking and anticipation about imagined or real future events (Davidson et al., 1990). The anxiety indicative behaviours seen in rodent models, such as failure to explore, increased thigmotaxis (contact with walls) have been extrapolated onto zebrafish in the highly successful novel tank test (Champagne et al., 2010). The fish are placed in separate tanks, and various parameters of behaviour are measured. In zebrafish, anxiety or anxiety-like behaviour is characterised by certain patterns of swimming, such as sticking to the walls of the tank and the bottom parts of tanks, reduced exploratory behaviour and longer time periods to swim to higher portions of the tank (Egan et al. 2009; Wong et al. 2010). Other patterns of behaviour include fast, erratic movements or bouts of freezing. Measurement of changes in these behavioural patterns helps in the establishment of an effective model to

study anxiety and the changes in levels of this anxiety-like behaviour on treatment with chemicals.

Purpose: In this study, the aim is to assess the anxiolytic effects of tea by exposing zebrafish to pure tea polyphenols as well as nanoparticles of tea polyphenols by comparing behavioural changes in the fish before and after treatment.

Methods: Behaviours such as anxiety are studied through various tests, such as the novel tank test and light/dark test. Fishes, when introduced into a novel tank, tend to swim towards the bottom. As time progresses, exploratory behaviour increases, and they transition to upper half of the tank (measured as latency to reach upper half). Another test used to measure anxiety through scototaxis is the light/dark test, where the tank is divided into to two vertical halves, one kept transparent (light) and one half blackened (dark). In our experiment, these two setups were used to check the anxiolytic effect of tea polyphenols, both in pure form and as nanoparticles. Measurements made for novel tank test include latency to reach the upper half (s), time spent in the upper half (s) and total number of transitions, recorded for a test period of ten minutes. For the light/dark test, the time spent in the light half was measured for a test period of ten minutes.

Results: We noticed decrease in the latency, and increase in the number of seconds spent in the upper half and increase in the number of transitions was seen in fish treated for ten minutes with the pure compound and nanoparticles in the novel tank test, as well as a decrease in the amount of time spent in the dark half of the tank in the light/dark test, while compared to the control.

Conclusion: Anxiety-like behaviour is characterised by reduction in exploratory behaviour, thigmotaxis and a preference of darker regions when introduced to a novel environment. In this study, changes in measurements of these behaviours were analysed in fishes given TPP when compared to those that were not given any treatment. It was found that there is indeed a pronounced overall decrease in erratic movements, thigmotaxis and freezing bouts when TPP was given. In most cases, a visual increase in exploratory behaviour, reduction in erratic movement and freezing were seen with increase in concentrations of the treatment. In some cases, the effects achieved with using TPP nanoparticles were comparable to that of treatment with direct TPP of same concentration (6.5 g/ml). In the case of time spent in light half of the tank, there is an increase with the nanoparticle treatment, and this shows that exposure to a gradual increase in concentration increases exploratory behaviour. The latency value however is also increased in the novel tank test. Visually, the fish was seen to explore the bottom of the tank languidly and then move to the upper half. These results denoting in anxiolytic property of TPP and increased exploratory behaviour in zebrafish is comparable to that of the effect of TPP in rodents. In the study conducted by Vignes, et al., 2006, mice treated with the green tea polyphenol epigallocatechin gallate showed increased exploratory behaviour that was seen to be dose dependent. This study also shows the importance of zebrafish as an animal behaviour model in both the study of the effect of compounds on behaviour as well as its success in comparison to rodent models.

The Systems Level Complexity of the Autism Spectrum Disorders

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Background: A disease that was once rare, autism has grown at alarming rates over the past many decades. It was believed for many years that there was a strong link between autism and the vaccination MMR (measles, mumps and rubella) which children receive within the first year of life, based on a study by Andrew Wakefield in 1998. Autism is a complex neurobehavioral condition that includes impairments in social interactions and developmental language and communication skills combined with restricted, repetitive behaviours. The epidemiology of ASD includes all racial, ethnic and socioeconomic groups, but is almost five times more common among boys than in girls. ASD is a disorder which comes under neurodevelopmental disorder. This serious disorder can seed into the inability to communicate and interact with people, which is also a chronic disorder. It can contribute phenotypic complexity due to numerous genetic factors. Genome-wide association studies (GWAS) has revealed many genes implicated in autism. Some of these genes are related to common biological pathways and gene networks. The alternative functioning of nervous cells and synapses can highly impact on neurodevelopment and information programming in the brain.

Purpose: The primary aim of the work was to identify the genetic link between neurodevelopment and neurodegenerative disorders from a systems perspective. We proposed to identify the molecular mechanisms that underlie the numerous conditions of neurodevelopment and neurodegeneration.

Methods: DisGeNET is a database with the largest available collections of genes and variants involved in human diseases. It gathers information from GWAS catalogues, expert curated repositories, animal models and the scientific literature. Gene Venn a web application was used for comparing gene lists using Venn diagrams, molecular and genetic interaction networks. Cytoscape and its plugins, namely, MCODE, BINGO, and JEPETTO were used for the various aspects of network construction, visualization and analyses. Disease – Connect using multiple resources.

Results: In our study, Disease Connect found link between PD, schizo and ASD. The first link was between the Parkinson disease (PD) and ASD and displayed a specific disease-gene relationship. The second link was between the

ASD and schizophrenia (SZ) and displayed the disease-gene relation. From the three significant pathways or processes the highest XD-score pathway is response to cocaine. Cocaine in all forms is the number one harmful drug of choice among pregnant women. All mothers used cocaine in one of its forms, polydrug use was common. Polydrug use ended with showing significant neurodevelopmental abnormalities in children including language delay in 94% of children and extremely high frequency of autism (11.4%).

Conclusion: The literature studies and gene network studies found that response to cocaine, neurotransmitter biosynthetic process and positive regulation of excitatory postsynaptic membrane potential are the common pathways for SZ, PD and ASD disorders. Cocaine abuse in adults might increase the risk of developing Parkinson's disease and schizophrenia, cocaine abuse of pregnant women increases the risk of developing autism in children. Thus, the ECM receptor interaction might be a common reason between the PD, SZ and ASD.

Understanding the Dystonia – Serotonin Connection: A Review of the Current Research Problems

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Background: Dystonia is a movement disorder in which muscles contract involuntarily, causing repetitive or twisting movements. The muscle spasms can be mild or severe and might interfere with the performance of day-to-day tasks. Dystonia affects about 1% of the population, and women are more prone to it than men. The condition can affect one part of the body (focal dystonia), two or more adjacent parts (segmental dystonia) or all parts of the body (general dystonia).

Purpose: To create awareness about the syndrome dystonia. Dystonias are clinically and genetically highly heterogeneous. Phenotypically, dystonias are classified as isolated, combined (in combination with another movement disorder), or complex dystonia (usually as one of several disease manifestations in a complex syndrome). Isolated dystonia can be caused by mutations in many genes, the most reported of which include – TOR1A (DYT1), TUBB4 (DYT4), THAP1 (DYT6), CIZ1 (DYT23), ANO3 (DYT24), and GNAL (DYT25). Combined dystonias (with Parkinsonism or myoclonus) are further subdivided into persistent (TAF1 [DYT3], GCHI [DYT5], SGCE [DYT11], ATP1A3 [DYT12]), PRKRA (DYT16), and paroxysmal (MR-1 [DYT8], PRRT2 [DYT10], SLC2A1 [DYT18].

Methods: 1. Is it dystonia? 2. Is it isolated or combined dystonia? – Are there other hyperkinetic components: what kind(s) of involuntary movements are present? – Are there hypokinetic components: what is the nature of any impairment of movement? Dystonia syndrome 1. What is the dominant movement disorder phenomenology? 2. What other movement disorders and other neurological features are present 3. What has been the temporal course of the disease – age at onset – sequence of development of neurological features – tempo of disease 4? What other systemic features are present 5. What does the brain imaging reveal?

Results: Isolated dystonia can be caused by mutations in many genes, the most reported of which include – TOR1A (DYT1), TUBB4 (DYT4), THAP1 (DYT6), CIZ1 (DYT23), ANO3 (DYT24), and GNAL (DYT25). Combined dystonias (with parkinsonism or myoclonus) are further subdivided into persistent (TAF1 [DYT3], GCHI [DYT5], SGCE [DYT11], ATP1A3 [DYT12]), PRKRA (DYT16), and paroxysmal (MR-1 [DYT8], PRRT2 [DYT10], SLC2A1 [DYT18]. Dystonia also can be a symptom of another disease or condition, including Parkinson's disease, Huntington's disease, Wilson's disease, traumatic brain injury and birth injury. It might involve altered nerve-cell communication in several regions of the brain. Some forms of dystonia are inherited. In dopa-responsive dystonias, genetic defects cause serotonergic functioning. In other inherited, acquired and idiopathic dystonias, disturbances of the serotonergic neurotransmission are reported.

Conclusion: The efficaciousness of serotonergicmedication furthermore suggests a shared pathophysiological mechanism of both motor andnon-motor symptoms in dystonia patients. Till today, serotonergic metabolism at the level of 5-HT or 5-HIAA has been studied in a limited number of predominantly genetic disorders. A better understanding of the pathophysiology of the different forms of dystonia and the involvement of the serotonergic system in motor as well as non-motor symptoms will enable more rational therapeutic strategies in dystonia.

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