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Meeting Abstracts

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1. Yeast studies on the benefit of simvastatin in reducing levels of amyloid betaian

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A large-scale epidemiology study on statins previously showed that simvastatin was unique among statins in reducing the incidence of dementia. Since amyloid beta (Aβ42) is the protein that is most associated with Alzheimer's disease, this study has focused on how simvastatin influences the turnover of native Aβ42 and Aβ42 fused with green fluorescent protein (GFP), in the simplest eukaryotic model organism, saccharomyces cerevisiae. Previous studies have established that yeast constitutively producing Aβ42 fused to GFP offer a convenient means of analyzing yeast cellular responses to Aβ42. Young cells clear the GFP fusion protein and do not have green fluorescence while the older population of cells retains the fusion protein and exhibits green fluorescence, offering a fast and convenient means of studying factors that affect Aβ42 turnover. In this study the proportion of cells having GFP fused to Aβ after exposure to simvastatin, atorvastatin and lovastatin was analyzed by flow cytometry. Simvastatin effectively reduced levels of the cellular Aβ42 protein in a dose-dependent manner. Simvastatin promoted the greatest reduction as compared to the other two statins. A comparison with fluconazole, which targets that same pathway of ergosterol synthesis, suggests that effects on ergosterol synthesis do not account for the reduced amounts of Aβ42 fused to GFP. The levels of native Aβ42 following treated with simvastatin were also examined using a more laborious approach, quantitative MALDI TOF mass spectrometry. Simvastatin efficiently reduced levels of native Aβ42 from the population. This work indicates a novel action of simvastatin in reducing levels of Aβ42 providing new insights into how simvastatin exerts its neuroprotective role. This reduction is likely to be due to protein clearance.

2. The segmented brain - why the hypothalamus is not part of the diencephalon and other surprises

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The traditional approach to classifying parts of the brain has been challenged by new findings on developmental gene expression. The columnar model espoused by Herrick in the early 20th century has been replaced by the prosomeric model of Puelles and Rubenstein (Puelles *et al.* TINS 2013;36:570). The prosomeric model shows that the brain is made up of a series of distinct segments - the hypothalamus (two segments), diencephalon (three segments), and midbrain (two segments), and the hindbrain (twelve segments).

The hypothalamus forms the rostral end of the neural tube and is divided into terminal and peduncular segments. The "new" diencephalon is divided into three rostro-caudal segments - the prethalamus, the thalamus, and the pretectal area. The midbrain, which is now included in the forebrain on gene expression grounds, can be divided into a large rostral segment (mesomere 1) and a much smaller pre-isthmic segment (mesomere 2). The hindbrain is also segmented, consisting of the isthmus and 11 rhombomeres (r1 to r11). In all areas of the brain, each segment contains all the dorsoventral elements of the neural tube: roof plate, alar plate, basal plate, and floor plate.

The most striking new features of the prosomeric model are seen in the hypothalamus. Firstly, it is now evident that the hypothalamus is not a part of the diencephalon (as was previously assumed), but a separate region which sits rostral to the diencephalon developmentally. The terminal segment of the hypothalamus forms the rostral end of the neural tube. The apparent ventral position of the hypothalamus (its name means "under the thalamus") in the adult brain is simply due to the sharp bend in the neural axis created by the cephalic flexure. This 180° bend even gives the illusion that the hypothalamus is continuous with the midbrain. Secondly, it is now evident that the subpallial and pallial components of the telencephalon are derived from the alar plate of the peduncular hypothalamus. The alar plate of the terminal hypothalamus gives rise into the preoptic area and the eye vesicle.

In the newly defined diencephalon, gene expression evidence now shows that the posterior commissure and related pretectal nuclei belong to the caudal diencephalon and not to the midbrain, as is popularly supposed. Within the hindbrain, the cerebellum arises from the alar plate of the isthmus and r1, and the pontine nuclei migrate from the alar plate of r6 to a ventral position in r3 and r4.

The traditional picture of brain anatomy has been based on a superficial interpretation of topography as seen in the adult human brain, without regard to the underlying ontological realities. The new discoveries concerning the underlying segmental nature of the brain give us a new understanding of the real interrelationships of brain structures. It will probably take years before the old formulations are abandoned; in the meantime it is important that medical students are presented with this new evidence, instead of being fed outdated ideas based on simplistic interpretations of brain topography.

3. The circadian system plays a major role in the aetiology, progression and treatment of parkinson's disease: ending an era of "forcing nature" with dopamine replacement

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There is an ever increasing body of research demonstrating that the circadian system plays an important role in the motor and non-motor symptoms of Parkinson's disease (PD). Historical evidence supporting this hypothesis can be found in the early work of Parkinson, Charcot and others, whereby the symptoms of this disease can vary in accordance with the phase of the circadian cycle. More recent work, examining the role of anatomical substrates of the circadian system, has shown that the retina, hypothalamus and pineal are important locations whereby underlying functional changes may well contribute to the aetiology of PD. Equally compelling are the recent preclinical and clinical findings demonstrating that more effective, less invasive therapeutic intervention may well be achieved if chronotherapeutics target these sub-anatomical parts of the circadian system. In our exploration these substrates we have identified the retina and the pineal as two such important locations where chronotherapeutics are most effectively delivered to produce the optimal therapeutic benefit while minimising adverse side effects. In particular, we will demonstrate how treatments, such as minute intravitreal injections of anti-PD drugs, produce robust therapeutic effects, that are normally attributed to deep brain structures. In contrast, the chronotherapeutic intervention observed in in the disease itself using strategic light therapy, provide additional evidence that circadian function plays a major role in the aetiology, progression and treatment of PD. It is time for a reappraisal of the underlying anatomical substrates reflexively attributed exclusively to the Nigro-striatal dopamine system. It is time to sojourn that endless search for the magic bullet of dopamine replacement and pursue the highly significant, but less invasive, contribution of chronotherapeutics in correcting this disorder.

4. Multicentric cryptococcomas mimicking neoplasia

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Fungal mass lesions in the central nervous system are, as a group, extremely rare. In this group, cryptococcomas are the most commonly seen and are often included in the differential diagnosis of the multicentric space occupying lesions in immunocompromised hosts. While crypococcomas are known to occur in both healthy and immunocompromised individuals, they are more commonly seen in the latter where *Cryptococcus neoformans* is the typical agent. This contrasts the species seen in immunocompetent hosts where *Cryptococcus gatti* occurs more commonly.

These lesions are commonly 3-10 mm in diameter and occur in the basal ganglia due to the organism spreading via the Virchow-Robbins spaces surrounding the small perforator vessels as part of contiguous spread from a basal meningitis. Although most frequently associated with HIV infection, patients with chronic renal disease, vascular conditions, hepatitis B or C, alcoholism, diabetes mellitus, and oncological diseases may also succumb to this infection and present with cryptococcomas.

In rare cases, a chronic granulomatous process may lead to formation of a mass lesion (cryptococcoma) that has a tumoral appearance. Metabolites released by *cryptococcus* can inhibit the migration and function of leukocytes and promote survival and localized replication of the pathogen, thus facilitating chronic granulomatous inflammation and giant cryptococcoma formation.

5. Analysis of repetitive element expression in the blood and skin of patients with parkinson's disease identifies differential expression of satellite elements

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Repetitive elements (RE) constitute the majority of the human genome and have a range of functions both structural and regulatory on genomic function and gene expression. RE overexpression has been observed in several neurodegenerative diseases, consistent with the observation of aberrant expression of RE posing a mutagenic threat. Despite reports that associate RE expression with Parkinson's disease (PD) no study has comprehensively analysed the role of these elements in the disease. This study presents the first genome-wide analysis of RE expression in PD to date. Analysis of RNA-sequencing data of 12 PD patients and 12 healthy controls identified tissue-specific expression differences and more significantly, differential expression of four satellite elements; two simple satellite III (repName = CAttC_nand_GAATG_n) a high-copy satellite II (HSATII) and a centromeric satellite (ALR_Alpha) in the blood of PD patients. In support of the growing body of recent evidence associating REs with neurodegenerative disease, this study highlights the potential importance of characterization of RE expression in such diseases.

6. Alzheimer's disease: is the inflammasome the missing link?

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Alzheimer's disease (AD) is a devastating neurodegenerative disease characterised by widespread neuronal cell death and progressive dementia. Genetic and molecular studies have confirmed the central role of amyloid-β (Aβ) production in the pathogenesis of AD. However, therapies eliminating Aβ from AD have unfortunately failed to stem progressive cognitive decline. The association of several immune responsive genes with increased AD risks have in recent years revealed that inflammatory mechanisms are also a powerful pathogenic forces in the process of neurodegeneration.

Inflammasome, a multiprotein complex, is implicated in the execution of inflammatory responses and pyroptotic death leading to neurodegeneration. Inflammasomes serve as platforms for the recruitment and activation of caspase-1, the de facto executioner of a diverse downstream inflammatory processes

including the maturation of two major pro-inflammatory cytokines, interleukin-1β (IL-1β) and interleukin-18. Increased IL-1β, a member of the IL-1 cytokine family, has been implicated in the response to Aβ deposition and up-regulated in specimens from patients with AD. Since IL-1β secretion is critically dependent on the activation of inflammasomes, inflammasomes have been inferred as the missing link for Aβ-induced IL-1β secretions.

Within the central nervous system (CNS), several types of inflammasome have been identified, of which the best characterised are the absent in melanoma 2, NOD-like receptor (NLR)-family pyrin domain-containing 1 (NLRP1), NLRP3 and NLR-family caspase recruitment domain (CARD)-containing 4 inflammasomes. Different subsets of inflammasomes contain different cytosolic pattern-recognition receptors and their assembly is initiated by different stimuli. Once activated, inflammasome induces an inflammatory cell death mode termed as pyroptosis. Pyroptosis is a process of programmed cell death closely associated with inflammasome activation. However, in contrast to apoptosis, in pyroptotic cell, the integrity of the cell membrane is affected and micro-pores are formed resulting in intracellular and extracellular ion imbalance cell swelling and rupture. Meanwhile, the pro-inflammatory cytokines are released to the extracellular space causing focal inflammation and cell death. Multiple potential targets upstream of pyroptosis signaling may pave the way for newly therapeutic drugs that may rescue inflammation in neurological diseases. This has incited us to study the response of human neurons to Aβ and to determine whether specific neuronal molecular events initiated link neuronal degeneration to an inflammatory response.

In our studies, Aβ was found to induce inflammasome activation and inflammasome-mediated pyroptosis. Using gene-trap mutagenesis approach, candidate genes, which could play an important role in regulating inflammasome-mediated pyroptosis have been identified. We also demonstrated that neural stem cells (NSCs) regulated the NLRP3 inflammasome, and inhibited the production of IL-1β and caspase-1 in activated microglia, as well as subsequently attenuating neurotoxicity caused by microglial neuroinflammation, adding to the inherent benefits of NSCs in AD treatment. By understanding precisely how inflammasomes work in the CNS under both physiological and pathological conditions, as well as determining how these inflammasomes can be pharmacologically targeted, we may be one major step closer towards developing a proper cure for AD.

7. Colocalization of iron and aluminum in nuclei of nerve cells in brains of patients with sporadic Alzheimer's disease

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The etiology of Alzheimer's disease (sporadic Alzheimer's disease, AD) remains to be clarified. However, growing lines of evidence indicate that metal-induced oxidative stress plays a key role in the pathogenesis of AD[1]. Recently, the presence of 8-hydroxydeoxyguanosine, a biomarker of oxidative DNA damage, was demonstrated in nuclear DNA (nDNA) in the AD brain. It has also been reported that accumulation of DNA damage is one of the earliest detectable events during the progression from healthy aging to dementia.

Iron (Fe) is a pro-oxidant metal capable of generating hydroxyl radicals that can oxidize DNA through Fenton reaction. Aluminum (Al) has been reported to facilitate Fe-mediated Fenton reaction, as shown in the chemical formulae below. These cyclic reactions continuously generate hydroxyl radicals and can cause severe oxidative damage to nDNA.

$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH\bullet + OH^-$ (Fenton reaction)
$Al^{3+} + O_2^-\bullet \leftrightarrow AlO_2^{2+}\bullet$ [2]
$Fe^{3+} + AlO_2^{2+}\bullet \rightarrow Fe^{2+} + Al^{3+} + O_2$ [2]

Since hydroxyl radicals are highly reactive, the half-lives of hydroxyl radicals have been reported to be as short as 10^{-9} s (1 ns). Therefore, to oxidize nDNA, Fe must be localized at close proximity to nDNA. To facilitate Fe-mediated oxidative reactions, Al must be colocalized at close proximity with Fe. However, the colocalization of Fe and Al in neuronal nuclei remains to be clearly demonstrated in the AD brain.

In this study, we examined the colocalization of Fe and Al in the nuclei of nerve cells in the AD brain using scanning electron microscopy (SEM) coupled with energy-dispersive X-ray spectroscopy (EDS). SEM-EDS analysis allows the concurrent imaging of subcellular structures with high spatial resolution and detection of small quantities of elements contained in the same subcellular structures.

Our results demonstrate that Al and Fe were colocalized in the nuclei of nerve cells in the AD brain. Within the nuclei, the highest levels of both Al and Fe were measured in the nucleolus. The SEM-EDS analysis also revealed the colocalization of Al and Fe in the heterochromatin and euchromatin in neuronal nuclei in AD brains. Notably, the levels of Al and Fe in neuronal nuclei in AD brains were markedly higher than those in age-matched control brains.

Additionally, it has been reported that metals, including Fe and Al, which bind to DNA or DNA-binding proteins, inhibit the repair of oxidatively damaged DNA. We hypothesize that the colocalization of Al and Fe in the nucleus of nerve cells might induce oxidative damage to nDNA and concurrently inhibit the repair of oxidatively damaged nDNA. An imbalance caused by the increase in DNA damage and the decrease in DNA repair activities might lead to the accumulation of unrepaired damaged DNA, eventually causing neurodegeneration and the development of AD.

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8. The role of high-flow bypass in the treatment of delayed complications of head and neck radiation

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Radiation therapy is the mainstay of treatment for head and neck cancers. Due to the prolonged survival of patients who have received radiation therapy, the incidence of delayed radiation-induced complications, including carotid stenosis and transient ischemic attacks, is expected to increase. Some of the survivors present with rare but devastating carotid blowout syndrome as a result of the ruptured carotid artery pseudoaneurysms. The best treatment of these delayed complications remains to be elucidated. We here

report our experience in treating seven patients with delayed radiation-induced complications using high-flow extracranial-intracranial bypass.

9. Spinal cord stimulation improves the microvascular perfusion insufficiency caused by critical limb ischemia

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Aim: The study aimed to identify the benefit and efficacy of spinal cord stimulation (SCS) in patients with perfusion problem caused by critical limb ischemia (CLI) compared with those who did not receive CLI.

Methods: Seventy-eight patients were diagnosed as having perfusion problem, with perfusion difference < 0.95, by using lower-limb 201TI scintigraphy. Thirty-seven patients were treated with SCS and 41 treated without. All patients took the same medications. The outcomes of walking distance, walking time, and sleeping quality were interrogated and recorded. The pain intensities were evaluated via visual analog scale score.

Results: The outcomes in SCS treatment group were dramatically ameliorated. The visual analog scale (VAS) score evidenced improvement immediately following one-week of SCS implantation. On the other hand, the outcomes in non-SCS group were exacerbated. Indeed, the increased intensities of microcirculation were observed in the lower extremities after SCS implantation compared with pre-implantation by using lower-limb 201TI scintigraphy. Most importantly, 10 of 41 patients were on wheelchairs in Non-SCS group, and no one on wheelchairs in SCS group after one-year follow-up.

Conclusion: Early diagnosis of perfusion problem in patients with CLI and treating with SCS immediately are crucial for the patients' improved outcomes and limb salvage.

10. Plasma biomarkers and neurodegenerative diseases

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Neurodegenerative diseases are now considered as proteinopathies of various combinations. Amyloid-β and tauopathies are the major pathognomonic pathological changes of Alzheimer's disease, α-synucleinopathies are for Parkinson's disease, and TDP-43 proteinopathies and tauopathies are for frontotemporal dementia. Other tauopathies include Pick's disease, corticobasal disease, and progressive supranuclear palsy. Synucleinopathies include multiple system atrophy and Lewy body disease. TDP-43 proteinopathies include amyotrophic lateral sclerosis. However, co-pathology of neurodegenerative diseases exist: Lewy bodies

commonly occur in Alzheimer's disease and Alzheimer's disease pathology is frequently found in Lewy body diseases, but the extent of such co-pathologies across neurodegenerative diseases remains undefined. The prevalences of proteinopathies of most neurodegenerative diseases were such that tau was nearly universal, amyloid-β was common, α-synuclein was less common, and TDP-43 was the least common. Recent development in cerebro-spinal fluid (CSF) biomarkers of neurodegenerative diseases demonstrated that tau proteins (both total tau and phosphorylated-tau) increased and amyloid-β (Aβ42) decreased in patients with Alzheimer's disease; α-synuclein increased in patients with Parkinson's disease; and so on. Nevertheless, the collection of CSF is invasive and is not without risk. Thus, blood-based biomarkers warrant further development. In our previous study, we developed a panel of plasma biomarkers by using immunomagnetic reduction assay technology including Aβ42, Aβ40, total tau, phosphorylated-tau, α-synuclein, phosphorylated α-synuclein, and TDP-43. Individual plasma biomarkers Aβ42 and total tau and combined biomarkers such as Aβ42/Aβ40 and Aβ42/tau performed well in differentiating older controls from patients with dementia due to Alzheimer's disease. α-synuclein and phosphorylated α-synuclein helped separate older controls from patients with Parkinson's disease, as well as assisted the differential diagnosis between Parkinson's disease and other atypical Parkinsonism. In this study, we demonstrated IMR assay results for Aβ42, total tau, phosphorylated-tau, α- synuclein, phosphorylated α-synuclein, and TDP-43 in five groups of patients including control (n = 39), mild cognitive impairment due to Alzheimer's disease (n = 40), dementia due to Alzheimer's disease (n = 34), Parkinson's disease (n = 28), and frontotemporal dementia (n = 30). We demonstrated the capacity of IMR blood-based (plasma) biomarkers in assisting diagnosis of individual neurodegenerative disease and showed the proteinopathy co-pathology in the blood.

11. Critical role of brain-specific gangliosides in the pathogenesis of traumatic brain injury and Alzheimer's disease

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Major brain glycosphingolipids, also called brain gangliosides, are localized within neuronal lipid rafts (NLR) of neuronal axons and synapses and their role in neurodegenerative diseases remains unknown. Here, we compared the outcome of traumatic brain injury (TBI) and Alzheimer's disease (AD) pathology in wild-type and glycosphingolipid-deficient animals. The *st3gal5* gene encodes for ST3 β-galactoside alpha-2,3-sialyltransferase 5, which is responsible for the biosynthesis of complex a, b, and c series gangliosides in the brain. We found that uninjured st3gal5-deficient mice exhibit normal cognitive and social behaviors, but also exhibit some very mild motor deficits. After TBI, st3gal5-deficient animals exhibit marked deficits in cognitive and motor functions, which was associated with increased hemorrhage and neuronal damage owing to the failure of NLR-induced platelet activation and serotonin secretion. The decrease in NLR-induced platelet-derived platelet activating factor release also resulted in reduced microglial activation and central nervous system macrophage infiltration in the st3gal5-deficient animals after TBI. Further investigation demonstrated that the interaction of platelets with NLR stimulated neurite growth, increased the number of dendritic spines, and increased neuronal activity during TBI. To understand the role of gangliosides in Alzheimer's disease pathology, we crossed st3gal5-deficient mice with 5XFAD transgenic mice that overexpress three mutant human amyloid proteins AP695 and two presenilin PS1 genes. We found that st3gal5-deficient 5XFAD mice had a significantly reduced burden of amyloid depositions, low level of neuroinflammation, and did not exhibit neuronal loss or synaptic dysfunction as compared to wild-

type 5XFAD mice. st3gal5-deficient 5XFAD mice also performed significantly better in a cognitive test than the wild-type 5XFAD control group. Finally, the treatment of wild-type 5XFAD mice with the sialic acid-specific *Limax flavus* lectin resulted in substantial improvement of AD pathology. Thus, our study establishes an important role for major brain glycolipids in the regulation of neuroinflammation, neuronal plasticity, synaptic functions, and cognitive ability after a neuronal injury during TBI- and AD-related neurodegeneration.

12. Analysis of the association of MIR124-1 and its target gene *RSG4* polymorphisms with major depressive disorder and antidepressant response

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Increasing evidence has indicated that dysfunction of miR-124 and target gene regulator of G protein signaling 4 (RGS4) may be involved in the etiology and treatment of major depressive disorder (MDD). However, the molecular mechanisms are not fully understood. This study aimed to investigate whether common genetic variations in these two genes are associated with MDD and therapeutic response to antidepressants in the Chinese population. Three polymorphisms including rs531564 [a functional single-nucleotide polymorphism (SNP) in MIR124-1], rs10759 (a microRNA-binding site SNP in RGS4), and rs951436 (a promoter SNP in RGS4) were genotyped in 225 Chinese MDD patients and 436 controls. Among the MDD patients, 147 accepted antidepressant treatment for eight weeks with therapeutic evaluation at baseline, Week 2, Week 4, Week 6, and Week 8 using the 17-item Hamilton Rating Scale for Depression. Multifactor dimensionality reduction (MDR) was used to identify gene-gene interactions. No significant association with MDD was discovered in single-SNP analyses. However, in the optimal model containing rs531564, rs10759, and rs951436 SNPs by MDR analysis, the *P*-value was 0.0024, the accuracy of the sample test was 0.49, and the cross-validation consistency was 10/10. Values of ORs and 95% confidence interval (CI) indicated that the combined action could increase the risk of MDD (OR = 1.67, 95%CI: 1.20-2.33). In pharmacogenetic study, a significant association was found in genotypic frequencies of rs951436 between the responder and non-responder groups (x^2 = 6.191, P = 0.045, correction P = 0.135) as well as between the remitter and non-remitter groups (x^2 = 7.216, P = 0.026, correction P = 0.078). For further analysis, the rs951436 heterozygote carriers had threefold probabilities of achieving clinical complete remission (OR = 3.00, 95%CI: 1.33-6.76, P = 0.007, correction P = 0.021) and 3.21-fold probabilities of achieving clinical response (OR = 3.21, 95%CI: 1.13-9.14, P = 0.022, correction P = 0.066) as compared with rs951436 homozygotes (AA + CC) after eight-week treatment. Moreover, the homozygous (AA + CC) of rs951436 showed a worse response to antidepressant treatment and had lower percent reduction of HAM-D scores over eight weeks than heterozygous AC, and significant associations were found at Week 6 (AA + CC *vs.* AC: 53.58 ± 27.04 *vs.* 61.34 ± 21.54, t = -2.08, P = 0.040) and Week 8 (AA + CC *vs.* AC: 60.17 ± 29.56 *vs.* 70.19 ± 20.41, t = -2.404, P = 0.018) after the adjusting for age and gender. In conclusion, an interaction effect of MIR124-1 and RGS4 polymorphisms may play a more important role than individual factors for MDD development. Moreover, RGS4 gene polymorphisms may be associated with antidepressant response among the Han population such as Weighted Correlation Network Analysis to explore pathogenic genes related to MDD, schizophrenia, and other psychiatric disorders. All directions of her research program are supported by the China National Major Project.

13. Ketogenic diets in parkinson's and alzheimer's

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Aging is accompanied by a mild decline in bioenergetic capacity on many structural levels (molecule, organelle, and cell) in cells throughout the body. In neurons afflicted by Parkinson's (PD) and Alzheimer's (AD), this decline is pathologically accelerated. Given that they alter multi-targeted nutrient sensors, metabolic therapies such as calorie restriction, intermittent fasting, and high-fat, low-carbohydrate ketogenic diets may be able to restore the bioenergetic decline at all these structural levels; ketogenic diets are probably the most sustainable of these options in PD and AD. In 2017, we developed a protocol to support 47 people with PD randomized to either a low-fat or ketogenic diet for 8 weeks. Primary outcomes were between-group changes in motor and nonmotor scores from baseline to week 8. By the end of the study, the ketogenic group showed clinically and significantly greater nonmotor score baseline improvements (41% compared to 11%), particularly in urinary problems, pain, fatigue, daytime sleepiness, and cognitive impairment. In mid-2019, Waikato Hospital will coordinate a similar randomized controlled study in patients with mild AD. Primary outcomes will be between-group changes in cognition, function, and quality of life scores from baseline to week 12.

14. Pharmacological annotation of polygenic risk in individuals with psychiatric disorders

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With high rates of heritability, the genetic analysis of psychiatric disorders is seen as an important strategy to identify the molecular determinants of its pathogenesis and therefore more specific targets for therapeutic intervention. In many respects, large genome wide association studies have delivered on this expectation, by revealing hundreds of genomic loci. Several significant challenges, however, remain to be overcome before we can more effectively capitalize on these discoveries, as most of the known genetic risk is complex and involves hundreds of genes. Where risk loci can be mapped to individual genes, their small effect size and/or low frequency may, by themselves, not present a compelling case for therapeutic development. To address this challenge, we are investigating an approach that exploits systems biology to aggregate genetic burden of complex traits into clinically actionable pathways. We have been exploring this concept with both SNP array and whole genome sequencing data for individual participants in the Australian Schizophrenia Research Bank cohort and identify several existing compounds that could potentially be directed with more biological specificity to patients with higher levels of risk in associated pathways. While some of these drugs have been used in schizophrenia, or are under investigation for use in the disorder, many are approved for use in other conditions and have not been considered in the context of psychiatric treatment. This approach has the potential to provide mechanism for precision treatment of schizophrenia and other psychiatric disorders, particularly in difficult treatment resistant cases.

15. Plasma-biomarker panel for discriminating Alzheimer's disease, Parkinson diseases, and Frontotemporal dementia

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Neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson diseases (PD), and Frontotemporal dementia (FTD) sometimes show similar change in one kind of biomarker as compared to healthy controls. Use of individual biomarker may cause low specificity to identify the disease type. The demand for a biomarker panel is growing to achieve a high-degree discrimination among different types of neurodegenerative diseases. In this work, the assay technology "immunomagnetic reduction" was applied to assay Amyloid Beta Peptide (Ab) 1-40, total Tau protein (T-Tau), phosphorylated Tau protein (p-Tau181), a-synuclein, phosphorylated a-synuclein (pS181), TDP-43, and Neurofilament Light (NfL) in human plasma for HC (n = 91), amnesic mild cognitive impairment (n = 41), AD (n = 35), PD with normal cognition (n = 47), PD dementia (PDD) (n = 62), and FTD (n = 25). For each kind of biomarkers, hematocrit (HC) shows a significantly lower level as compared to disease groups. It was found that FTD shows the highest levels of T-Tau (41.5 ± 20.5 pg/mL), p-Tau181 (6.67 ± 1.34 pg/mL), and TDP-43 (0.356 ± 0.202 pg/mL). AD shows the highest levels of Ab1-42 (21.2 ± 7.2 pg/mL). PDD shows the highest levels of a-synuclein (4.76 ± 1.10 pg/mL) and pS181 (12.4 ± 18.6 fg/mL). According to these data, a plasma-biomarker panel constructed with Ab1-42, T-Tau, and a-synuclein is promising for differentiating AD, PD, and FTD.

16. Circulating circular RNAs as biomarkers in the acute phase of ischemic stroke

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Currently, there are no valuable blood-based biomarkers that can be used for diagnosing acute ischemic stroke (AIS) and predicting stroke outcomes. circRNAs show promise as stroke biomarkers because of their participation in various pathophysiological processes associated with stroke and stability in peripheral blood. To explore circulating circRNAs associated with AIS, their utility as an early diagnostic marker and their significance in predicting stroke outcomes, a circRNA microarray was used to identify differentially expressed circulating circRNAs in a discovery cohort of three patients with AIS and three matched healthy control subjects (HCs). Validation was performed in an independent validation cohort (36 patients with AIS and 36 matched HCs) by quantitative real-time polymerase chain reaction (qRT-PCR). The replication cohort (200 patients with AIS and 100 HCs) was used for large sample verification, and the

copy numbers per microliter plasma were calculated by qRT-PCR. We identified, validated, and replicated three differentially expressed circRNAs, which were upregulated in patients with AIS compared with HCs (circFUNDC1: $P = 0.00014$; circPDS5B: $P = 4.13 \times 10^{-9}$; circCDC14A: $P = 1.86 \times 10^{-9}$). With an area under the curve (AUC) of 0.875 corresponding to a specificity of 91% and a sensitivity of 71.5%, the combination index of these three circRNAs had diagnostic power for stroke. The baseline circRNA levels showed poor significance, but the change rate in the level of circRNAs within the first seven days of treatment showed significance in predicting stroke outcomes (AUCs of circFUNDC1, circPDS5B, circCDC14A, and the overall circRNA set were 0.884, 0.953, 0.943, and 0.960, respectively). The elevation levels of circRNAs after stroke might be due to increasing levels in lymphocytes and granulocytes. In conclusion, a set of circulating circRNAs - circFUNDC1, circPDS5B, and circCDC14A - could not only serve as biomarkers for AIS diagnosis but also be applied in predicting stroke outcomes.

17. Stress-induced changes of NMDA and AMPA receptor expression in the rat brain are aggravated by neonatal bacterial endotoxin exposure

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Deregulated glutamatergic transmission is known to be implicated in neurological-psychiatric disorders, including stress-evoked schizophrenia and post-traumatic stress disorder (PTSD). Structural changes of NMDA and AMPA receptors can affect glutamatergic transmission. These receptors have a heterotetramer structure: NMDA-Rs consist of obligatory GluN1 subunit and variable GluN2 (a-d) or GluN3 (a and b) subunits; AMPA-Rs are composed of obligatory GluA2-dimer and a dimer of two other subunits, GluA1, GluA3, or GluA4. The expression of NMDA-R and AMPA-R subunit genes in regards to vital stress has only been explored in few studies without investigation of long-term changes, even though this seems important for understanding the mechanisms of PTSD. Moreover, these disturbances of glutamic receptor subunit expression are hypothesized to become even more vulnerable to stress effects after early-life immune challenges. According to the "two-hit" hypothesis, neonatal pro-inflammatory activation ("first hit") can affect brain maturation, thus making stressful events later in life ("second hit") have a more pronounced negative effect on brain function that can cause severe mental disorders, including schizophrenia.

The present study was aimed at the investigation of NMDA-R and AMPA-R subunit gene expression in the rat brain in a model of vital stress alone or combined with neonatal lipopolysaccharide exposure.

Two series of experiments were performed: male three-month-old Wistar rats were subjected to stress associated with contact with a predator (a black-tailed python) for 40 min either without neonatal manipulation (Study I) or after treatment with lipopolysaccharide (LPS), 25 or 50 μg/kg, i.p., at P15, P18, and P21 (Study II). qRT-PCR analysis of mRNA expression of NMDA (GluN1, GluN2a, and GluN2b) and

AMPA (GluA1 and GluA2) glutamate receptors was performed in the brain structures of rats at 6 or 24 h, 3, 9, and 25 days after stress for Study I, and seven days after stress for Study II.

In Study I, the most pronounced alterations of gene expression were revealed 25 days after stress: mRNA level of GluN2a NMDA-R subunit was upregulated in the amygdala of stressed animals compared to non-stressed control; GluN2b expression increased in the ventral hippocampus (VH) and medial prefrontal cortex and decreased in the dorsal hippocampus (DH) of rats exposed to vital stress in comparison with control. Expression of GluA1 and GluA2 decreased in DH and increased in VH after stress.

In Study II, mRNA expression of GluN2a, GluN2b, and GluA2 subunits was upregulated in mPFC of non-stressed animals injected with 25 μg/kg LPS. Levels of GluA1 subunit mRNA in DH of LPS-treated (50 μg/kg) rats increased, with no changes in VH of non-stressed LPS-treated animals. Stress-induced changes were more prominent in animals injected with 50 μg/kg LPS. In mPFC of stressed LPS-treated rats, when compared with vehicle-treated control groups, the levels of GluN1, GluN2a, GluN2b, GluA1, and GluA2 mRNA, as well as GluN2a/GluN2b ratio, were increased, while, in DH, GluN2a and GluA1 subunit mRNA levels were downregulated, and GluN2a/GluN2b ratio decreased.

Thus, early-life LPS treatment aggravates stress-induced disturbances of NMDA-R and AMPA-R subunit expression, which may contribute to severe mental illnesses.

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18. The pathogenic role of complement C5a receptor, C5aR1 in motor neuron disease

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The complement system is upregulated in MND, with recent studies indicating that the activation product C5a may accelerate disease progression via its receptor, C5aR1. This study examined the pathological role of C5aR1 in $SOD1^{G93A}$ mice, using $SOD1^{G93A}$ mice lacking C5aR1 and by means of pharmacological inhibition of C5aR1 using PMX205. C5aR1 deficient mice were backcrossed to $SOD1^{G93A}$ mice to generate $SOD1^{G93A}$ mice lacking C5aR1. The selective and orally active C5aR1 antagonist, PMX205, was also administered to $SOD1^{G93A}$ mice via their drinking water, both pre- and post-disease onset. The effect of C5aR1 genetic ablation and/or pharmacological inhibition using PMX205 on disease progression of $SOD1^{G93A}$ mice was determined using body weight, hind limb grip strength, survival time and molecular analysis of spinal cord, tibialis anterior and blood. $SOD1^{G93A}$ mice lacking C5aR1 and $SOD1^{G93A}$ mice treated with PMX205 prior to disease onset, both had significantly improved hind-limb grip strengths, slower disease progression and extended survival, compared with control or vehicle treated $SOD1^{G93A}$ mice. These improvements in the $SOD1^{G93A}$ mice lacking C5aR1 and PMX205-treated group were associated with reductions in pro-inflammatory monocytes/macrophages/microglia in the peripheral blood, tibialis anterior and spinal cord. There was also a reduction in pro-inflammatory cytokines in the lumbar spinal cord. Importantly, PMX205 treatment beginning several weeks following disease onset also had an attenuating effect on disease progression, significantly extending survival. These results confirm that C5aR1 plays a pathogenic role in

$SOD1^{G93A}$ mice, further validating the C5a-C5aR1 signalling axis as a potential therapeutic target to slow disease progression in MND.

19. Gap junction network within an olfactory sensory unit for colony identification in the Japanese carpenter ant: 3D structure and putative function

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The environment is filled with chemical information, and animals, including human beings, have developed adaptive chemosensory systems. It is thought that the chemical information is integrated in the brain before making a decision. Here, I talk about a complicated olfactory sensory system of a tiny insect, the carpenter ant, *Camponotus japonicus*, because we recently found an information integration network at this very peripheral system. For colony identification, worker ants utilize a colony-specific body odor consisting a characteristic blend of cuticular hydrocarbons (CHCs) as a social pheromone. C. *japonicus* workers appeal to their own colony identification with the colony-specific body odor comprising 18 species-specific CHCs. Thus, the accurate difference detection among such colony-specific body odors of workers is indispensable for their social life while inaccurate difference detection is sometimes fatal in competition among colonies. The body odor CHCs is sensed in a particular type of olfactory organ called Sensilla basiconica on the antennae. The number of *S. basiconica* responding to own colony's CHCs was significantly smaller than that responding to other colony's CHCs. This suggests that the very peripheral tiny sensory system possesses a whole basic machinery for colony identification via odor difference detection. To investigate the functional design of this type of sensilla, we observed its ultra-structures, using a serial block-face scanning electron microscope (SBF-SEM). Based on the serial images of 352 cross sections of SBF-SEM, we reconstructed a 3D model of the sensillum. This model reveals that each S. basiconica houses > 100 unbranched dendritic processes, which extend from the same number of olfactory receptor neurons (ORNs). The dendritic processes have characteristic beaded-structures and form a twisted bundle within the sensillum. At the beaded-structures, the cell membranes of the processes are closely adjacent in the interdigitated profiles, suggesting functional interactions via gap junctions (GJs). Immunohistochemistry with anti-innexin (invertebrate GJ protein) antisera revealed positive labeling in the antennae of C. *japonicus*. Innexin 3, one of the five antennal innexin subtypes, was detected as a dotted signal within the S. basiconica as a sensory organ for colony identification. The fluorescence intensity of innexin 3 shows a characteristic twin-peak-distribution similar to the distribution of adhesion regions at beaded-structures. These morphological results suggest that the beaded-structure provides a platform for functional connection among ORNs via close apposition of membranes and ORNs form an electrical network via GJs between dendritic processes. To reveal the function of the ORNs network via GJs, we examined a simplified mathematical simulation for the inter-dendritic neural network based on cable theory and proposed possible modification of its responsiveness to virtual stimulation. The mathematical simulation showed that the information network acts as a "stronger-input-spread or weaker-input-cut filter" in a GJ-distribution-dependent manner. This novel "filter" supports that ORNs in the S. basiconica generate few impulses when they respond to own colony's CHCs (weak stimulation) and generate many impulses when they respond to other colony's CHCs (strong stimulation). Therefore, the ORN network via GJs possibly contributes to the distinct identification of colony-specific blends of CHCs.

20. Tissue-engineered electrodes for brain-machine interfaces

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State-of-the-art neural interfaces rely on conventional metallic electrodes. Ideally, bionic devices should safely operate for a lifetime; However, the fibrotic tissue encapsulation leads to inefficient stimulation and formation of toxic by-products, ultimately compromising the electrical and biological performance of these bionic interfaces. This limitation further challenges the development of smaller and more densely packed electrodes aiming for a more specific neuronal stimulation. Conductive hydrogel (CH) coatings, based on poly (vinyl alcohol) polymers modified with conductive polymers, can provide enhanced electrical properties, superior to those of traditional platinum (Pt) electrodes. These coating materials can be further modified to include an overlaying layer of neural progenitors encapsulated within a 3D biosynthetic hydrogel. This study tested the hypothesis that a CH coated electrode decorated with a loaded cell coating provides a more physiological interface able to integrate electrodes with the neural tissue without significantly reducing the charge transfer properties. The aim of this study was to develop and assess a tissue-engineered, living electrode (LE) coating for brain-machine interfaces. LEs were fabricated by first coating intra-cortical Pt electrodes with CH followed by an overlaying degradable bio-synthetic hydrogel coat loaded with primary neural progenitor cells. The electrical performance of LEs was compared with conventional Pt electrodes *in vitro* and *in vivo*. *in vitro* studies confirmed that overlaying a neural cell-loaded coat on the CH did not significantly impacted the electrode impedance and charge storage capacity. These results suggest that the electrical performance of LEs was comparable to standalone CH coated electrodes and significantly superior to Pt electrodes. *In vivo* studies showed that implanted LEs and uncoated Pt electrodes in a rat brain model did not cause any adverse events over 8 weeks. LEs presented a significantly higher signal to noise ratio than Pt electrodes. On-going research is assessing the tissue response to implanted LEs. These results demonstrate the potential for LEs to support the development of more robust neural interfaces.

21. Golgi fragmentation induced by cyclin-dependent kinase 5 overactivation is associated with isoflurane-induced cognitive decline

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Isoflurane is a widely used anesthetic. Isoflurane exposure induces cognitive decline, especially in elderly patients, while the underlying mechanism remains to be elucidated. In the present study, we explored whether Golgi fragmentation is relevant to isoflurane-induced cognitive decline and the underlying molecular mechanism in aged mice. Sixteen-month-old C57BL/6J mice inhaled 1.4% isoflurane for 2 h daily for three consecutive days. To inhibit aberrant cyclin-dependent kinase 5 (Cdk5), 10 mg/kg roscovitine was given 30 min before isoflurane treatment. The Golgi structure, Cdk5 activity, and level of p25/p35 were assessed 2 h after isoflurane exposure. Spatial learning and memory ability of mice were evaluated

by Morris water maze one day after isoflurane treatment. Our results show that the number of fragmented Golgi and Cdk5 activity increased. Learning and memory ability were impaired in aged mice after isoflurane exposure, while Cdk5 inhibitor roscovitine rescued the Golgi structure and improved learning and memory performances. In addition, after isoflurane exposure, the levels of p25/p35 increased, while Cdk5 levels unchanged. Our study reveals that the cleavage of p35 into p25 may contribute to aberrant Cdk5 activation, and Cdk5 overactivation-induced Golgi fragmentation may mediate isoflurane-induced cognitive decline in aged mice. Inhibition of aberrant Cdk5 activation alleviates Golgi fragmentation and cognitive decline, which provides a potential therapeutic approach for isoflurane-induced cognitive decline.

22. A novel analytical method for detection of phosphorylated a-synuclein S129 in Parkinson's disease

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Parkinson's disease (PD) is characterized by the intraneuronal α-synuclein inclusions called Lewy bodies. Increase of phosphorylation of a-synuclein in Serine 129 (pS129) has been correlated with the aggregation, toxicity, protein interaction, and turnover of α-synuclein. Thus, pS129 can indicate the pathogenesis of PD. Since the concentration of pS129 in the plasma (femtogram level) is far lower than the normal detection range of ELISA, we developed an ultrasensitive immunomagnetic reduction assay to detect the trace amount of pS129 in limited volume of human plasma (60 μL). The pS129 assay covered a range of concentration (0.00048-144.78 pg/mL) with a limit of detection of 0.065 fg/mL. Furthermore, we analyzed the pS129 level from healthy control (n = 10) and patients with PD (n = 23) and found a significant increase of pS129 in plasma from PD ($P < 0.0001$). The cut-off value of pS129 for discriminating control from PD was 0.505 fg/mL with corresponding clinical sensitivity and specificity of 95.65% and 100%, respectively. In conclusion, we developed a novel plasma pS129 assay that is convenient, sensitive, sample saving, and useful for identifying PD patients.

23. Linking autism to an imbalanced catabolism of synaptic monoamine

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An interdisciplinary study of autism led to implicate a relatively poor catabolism of one of the monoamines released in the synapse, namely *serotonin*. This deficit would result from persistent epigenetic regulations of two enzymes (i.e., MAOA- and COMT+) across neural differentiation, for counteracting an accidental excess of MAOA in the early gestation. Epigenetic traits would outlast this temporary excess and be inherited by generations of neurons, and possibly by next human generations. In addition, the late occurrence of autistic symptoms may be consistent with the increase of the monoamine oxidase B (MAOB)

enzyme that degrades another monoamine (dopamine), but only significantly around two years after birth. The consequent long-term imbalance of synaptic monoamines is assumed here to impact the architecture of sleep and learning[1], inducing a range of developmental problems.

This theory is drawn on Guided Propagation Networks (GPNs), the computer simulations of which show the growth of aberrant structures when modulation parameters akin to monoamines do not satisfy inner learning constraints. Comparisons are made between a reference well-tuned network and others grown with shifted parameters, all using the same learning data. Unlike the reference network, impaired GPNs display features that have been observed in the autistic brain: (1) more local connections (here underlying either repetitive behavior or over-activity); (2) missing or impaired long-distance connections (which convey emotional conditioning towards decision-making modules); and (3) overgrowth: the overall connectivity can involve 1.5 more cells and links. Apart from these computer experiments[2], the 4:1 sex ratio observed in autism can be calculated in a family tree which combines genetic variants and epigenetic regulations. According to this calculation, which involves two types of genetic masking of the relevant epigenetic traits (i.e., X-silencing and low-COMT), in addition to 1.5% of the population having developed an overt form of autism, about 6% of men and 24% of women would be "healthy carriers" of the enzymatic (dys) regulation at issue.

On the medical side, an epileptic 11-year-old boy with severe autism received sodium valproate daily for its ability to both stimulate MAOA and treat epilepsy. In this case study, behavioral changes have been recorded for one year by parents and caregivers unaware of the autism target. This one-year monitoring showed improvement of sleep and then gaze, followed by a gradual decrease of stereotypy among other behavioral changes arising nine months after the treatment initiation. Hyperactivity, which hindered learning across this treatment, could afterwards be reduced by low-dose of the methylphenidate psychostimulant. The proposed dual therapy thus involves a MAOA inducer and a psychostimulant, together with re-education, all monitored by relevant biomarkers. If validated by future investigations, this approach is first intended to prevent early gestation from environmental factors that are likely to stimulate the production of MAOA, including small-sized fatty acids.

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24. Neuropsychology intervention in Williams syndrome: a clinic case

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Williams syndrome (WS) is a neurodevelopmental disorder caused by the removal of 7q11.23. Patients with WS present neuroanatomical alterations that are reflected in neuropsychological alterations mainly in visuospatial abilities, attention, and executive functions. The objective of the study was to apply a neuropsychological intervention program to improve attention and visuospatial skills. The program was applied for 10 months in sessions of 1.5 h to an 8-year-old girl with WS from Mexico City. The tasks were designed based on neuropsychological clinical models. The pre- and post-intervention results were compared with a clinical sample of five patients with WS, which allowed identifying if there were an

improvement and clinical recovery. The results were analyzed using the reliable change index. The results reveal that the patient improved her intellectual abilities, attention, and visuospatial abilities. They also improved the skills of abstraction and memory. In the literature, it is related that the neuropsychological intervention stimulates the activation of alternative or inactive neural networks that begin to have a greater implication in the affected cognitive processes. These findings demonstrate that neuropsychological intervention is an effective therapeutic strategy for patients with congenital brain damage.

25. Caspr2/CNTNAP2 (or cadm1) forms a complex with GPR37 and Mupp1 but not with autism-related mutated ones

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Autism spectrum disorder (ASD) is one of the developmental brain disorders. Mutations in the synaptic components including NLGN, Nrx, Cadm1, Caspr2/CNTNAP2 (Contactin-associated protein 2), and GPR37 (G-protein-coupled receptor 37) have been found in ASD patients. Caspr2 and Cadm1 have a PDZ binding domain at C-terminal region and form a complex with receptors via interaction with Multiple PDZ domain protein 1 (Mupp1). However, little is known about the impaired Caspr2 (Cadm1)-Mupp1-receptor complex related to the pathogenesis of ASD. Recently, we found mutations (R558Q and Del312F) of GPR37 gene in the ASD patients. Caspr2 (Cadm1) and GPR37 mainly interacted with PDZ3 and PDZ11 domains of Mupp1 via their C-terminal PDZ binding domains, respectively, while the ASD-associated mutated GPR37 (R558Q) more weakly interacted with Mupp1 and was much less transported to the cell surface by Mupp1. In the present study, we found two missense mutations in *Mupp1* gene of the ASD patients and investigated the density and morphology of PSD95-positive dendritic spines and protrusions in cultured hippocampal neurons overexpressing the mutated Mupp1. Compared to the Mupp1, mutated Mupp1 significantly reduced the density of PSD95-positive dendritic spines and decreased the width of dendritic spines. Thus, ASD-related Mupp1 missense mutations influence the dendritic spine morphogenesis, causing the pathogenesis of ASD. The impaired Caspr2 (Cadm1)-Mupp1-receptor complex including Gpr37 or serotine receptors may be related to the pathogenesis of ASD.

26. The prognostic factors affecting the occurrence of subsequent unprovoked seizure in patients who present with febrile seizure after 6 years of age

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Aim: Few reports have described the prognostic factors affecting the occurrence of subsequent unprovoked seizure in patients who present with febrile seizure (FS) after six years of age. We investigated the prognostic factors affecting the development of unprovoked seizures after FS among patients from Jeju Island.

Methods: We included patients who developed FS after six years of age and presented to our outpatient clinic between January 2011 and June 2017. Clinical data were obtained through chart reviews and phone call interviews. We used logistic regression analysis to analyze the risk factors associated with the occurrence of subsequent unprovoked seizure.

Results: Of the 895 patients, 83 developed FS after six years of age. Among these 83, three patients were prescribed antiepileptic drugs before the onset of the unprovoked seizure and four patients developed an unprovoked seizure before six years of age. Thus, overall, 76 patients were included in the study. Fifty-one patients developed first FS before six years of age. In the remaining patients, the first FS developed after six years of age. The mean observational period since the last outpatient follow-up visit was 3.2 years (median 3.04 years, range: 1.42-4.71 years). Among them, 21% developed an unprovoked seizure. Logistic regression analysis showed that electroencephalographic (EEG) abnormalities serve as an independent risk factor for a subsequent unprovoked seizure.

Conclusion: EEG is the proper diagnostic tool to predict the risk of a subsequent unprovoked seizure in patients with FS after six years of age.

27. Comparative effects of hydrogen sulfide-releasing compounds on [3H]D-aspartate release from bovine isolated retinae

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Previously known as an industrial toxicant, hydrogen sulfide (H_2S) has evolved into a signaling gasotransmitter that possess physiological roles in the central nervous, cardiovascular and the immune systems (Kimura Molecules 2014;19:16146). It is endogenously derived from *L*-cysteine and *D*-cysteine by cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE) enzymes, with some contribution from cysteine aminotransferase and *D*-aminotransferase in combination with 3-mercaptosulfurtransferase using *L*-cysteine or homocysteine as substrates (Abe & Kimura. J Neurosci 1996;16:1066; Nagai Y *et al*. FASEB J 2004;18:557). In ocular tissues, a deficiency of CBS due to a mutation in the gene encoding the enzyme is associated with several eye disorders such as glaucoma, cataracts and retinal detachment [Kraus JP, Kozich V. In Carmel & Jacobsen DW (eds) Homocysteine in health and disease. Cambridge University Press; 2001. p. 223]. Furthermore, H_2S has been shown to exert pharmacological effects on mammalian ocular tissues from both anterior and posterior segments *in vitro* and *in vivo* (Ohia *et al*. J Ocul Pharmacol Ther 2018;34:61). In the present study, we used the Superfusion Method to (1) compare the pharmacological actions GYY 4137, a slow-releasing H_2S donor to that of *L*-cysteine, a substrate for H_2S biosynthesis on K^+-evoked [3H]D-aspartate release, and (2) examine the role of KATP channels and nitric oxide (NO) in the responses elicited by these compounds on neurotransmitter release in isolated bovine retinae. GYY 4137 (10 nM - 10 μM), *L*-cysteine (100 nM - 10 μM) and its prodrug, *N*-acetyl cysteine (10 μM -1 mM) attenuated K^+-evoked [3H]D-aspartate release in a concentration-dependent manner from isolated bovine retinae without affecting basal tritium efflux. The rank order of activity observed at an equimolar concentration of 10 μM was: *L*-cysteine > GYY 4137 > *N*-acetyl cysteine. Interestingly, the dual inhibitor of the biosynthetic enzymes for H_2S, CBS and CSE, amino-oxyacetic acid (3 mM) reversed the inhibitory

responses caused by the three H2S donors on K^+-evoked [3H]D-aspartate release. Glibenclamide (300 μM), an inhibitor of KATP channels reversed the inhibitory action elicited by GYY 4137 and *L*-cysteine but not that of *N*-acetyl cysteine on K^+-induced [3H]D-aspartate release, suggesting a distinct and unique mechanism for the *L*-cysteine prodrug. The inhibitory effect of GYY 4137 and *L*-cysteine on neurotransmitter release was reversed by the non-specific inhibitor of NO synthase (NOS), L-NAME (300 μM). Furthermore, a specific inhibitor of inducible NOS, aminoguanidine (10 μM) mitigated the inhibitory action of *L*-cysteine on K^+-evoked [3H]D-aspartate release. We conclude that both donors and substrates for H2S production can inhibit amino acid neurotransmission in bovine isolated retinae, an effect that is dependent, at least in part, upon the intramural biosynthesis of this gas, and on the activity of KATP channels and NOS enzyme (Bankhele *et al*. Neurochem Res 2018;43:692).

28. Surgical outcome of pediatric spinal cord tumor

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Aim: The objective of this study was to examine the long-term surgical outcomes of pediatric spinal cord tumor in a prospective multicenter database.

Methods: Of 48,901 surgical cases in our database, 1046 (2.1%) involved patients under 20 years old. Among these, 47 cases (0.1%; male 28, female 19; mean age 11.1 years; and mean follow-up: five years) were spinal cord tumors with clinical records, plain radiographs, and MRI. The patient characteristics, symptoms at onset, tumor resection, surgical procedure, postoperative radiotherapy and chemotherapy, surgical outcome, and kyphotic change at final follow-up were examined. Statistical analysis was performed by unpaired t-test and Fisher exact test.

Results: Intradural extramedullary, intramedullary, and extradural tumors accounted for 50%, 33%, and 17% of the 47 cases, respectively. A thoracic spine tumor was most common (40%). The common pathological diagnoses were ependymoma (n = 8), neurinoma (n = 7), and neurofibroma (n = 6), including high-grade malignant spinal tumor. The most common symptom at onset was pain (50%), followed by motor palsy (34%), gait disturbance (18%), and bladder disturbance (15%). In 35% of the cases, pain was the only preoperative symptom. Total resection was achieved in 61% and subtotal resection in 22% of cases, and radiotherapy and chemotherapy were performed postoperatively in 18% and 14%, respectively. The recurrence rate was 24%, and these cases were treated with additional surgery and chemotherapy. Postoperative improvement of symptoms occurred in 38 cases (81%), but there were four deaths due to a malignant tumor. Progression of spinal kyphosis (> 5°) occurred in 18 cases (38%), with an average of 11°. Postoperative kyphosis was significantly related to postoperative radiation therapy ($P < 0.05$), but not to the number of laminectomy levels.

Conclusion: In pediatric spinal tumor, the main symptom at onset was pain without neurological deficit. Postoperative radiotherapy may be effective, but postoperative kyphotic changes are a concern in these patients.

29. The Di Bella method's biological multi-therapy has improved the objective response, expectation and quality of life of brain malignancies: Statistical evaluation at seven years

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The interaction between growth hormone (GH) and prolactin receptor (PRL) acts on physiological and neoplastic growth, which uses these factors on multiple physiological measures, with dose-dependent relationship. From a review of the literature, GH and growth hormone receptor (GHR) overexpression in tumors is constant. In more than a thousand cases published on www.pubmed.gov by Giuseppe and Luigi Di Bella of various neoplasms, an improvement in the objective response, quality of life, and survival was documented, compared to conventional oncological protocols, inhibiting GH and GF correlated through somatostatin and analogs in the context of biological multi-therapy "Metodo Di Bella" (MDB).

Rationale-Mechanism of action of therapy. The Di Bella Foundation has been treating and monitoring for nine years several cerebral neoplasms including oligodendrogliomas 2° and 3°, astrocytomas, glioblastomas 3°, anaplastic gliomas 2° and 3°, and anaplastic oligoastrocytomas.

- Somatostatin 3 mg - subcutaneous (every night - 12 h infusion)
- Slow Octreotide 20 mg intramuscular release (every three weeks)
- Conjugated Melatonin (Melatonin 12%, Adenosine 51%, and Glycine 37%) 100 mg (daily-oral)
- Retinoid solution - 8 mL oral (three times a day) (ATRA 0.5 g, Axeroftole Palm. 0.5 g, Beta Carotene 2 g, and Alpha Tocopherol Acet. 1000 g)
- Vit. D3, 1.25; OH-Tachysterol, 10-12 drops (three times a day - oral)
- Tetracosactide Acetate 0.25 mg intramuscular (three times a week)
- Cabergoline 0.5 mg (½ cps) - oral (twice a week)
- Bromocriptine 2.5 mg (½ cps) - oral (twice a day)
- Temozolamide (20 mg/morning and evening) daily metronomic administration (morning and evening) Hydroxyurea 500 mg at midday meal
- Ac. Slow release valproic 500 mg morning and evening
- Calcium Levofolinate capsules 22 mg per day
- Vit. C 2 gr (2 times a day)

Somatostatin + Octreotide with antiproliferative function and receptor saturation are interactive with dopamine 2 receptor (D2R) agonist prolactin inhibitors, whose PRLR receptor is co-expressed with GHR on cytosolic membranes. MDB multi-therapy with inhibition of GH-PRL proliferative axis and GH-dependent growth factors (IGF1-FGF-VEGF-EGF) with anti-proliferative effect have increased life expectancy, on average over six years, in the mentioned neoplasms.

The myelotoxicity of the metronomic use of Temozolamide-Hydroxyurea is contained by the myeloprotective properties of melatonin and retinoid solution, which, with vitamins D3 and C, exert a differentiating synergism, with antioxidant and immunomodulating activities; instead, with somatostatin and prolactin inhibitors, they exert a cytostatic effect. For the still reduced survival of malignant brain tumors (Glioblastoma, 15 months), we consider useful this biological multi-therapy of synergistic and factorially interactive molecules, singularly managed by non-toxic antitumor activity, which act centripetally on the myriad biological reactions of the tumoral life, bringing back to normal the vital reactions deviated due to cancer.

30. Thrombolysis with rhTNK-tPA at different therapeutic time windows in animal model of embolic stroke

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It is essential for us to investigate the effects and characteristics of thrombolytics on different therapeutic time windows (TTW) in an animal model of embolic stroke. In our research, we investigated the thrombolysis with recombinant human TNK-tPA (rhTNK-tPA) on thromboembolic stroke in an animal model at different TTW. Rats were subjected to embolic middle cerebral artery occlusion. RhTNK-tPA and positive control drugs rt-PA were administered 1, 2, 3, 4.5, and 6 h after inducing thromboembolic stroke. Neurological deficit scoring (NDS) was evaluated at 6 and 24 h after the treatment. The lesion volume in cerebral hemispheres was measured by MRI using MRI scanning machine after 6 h of thrombolysis, and the infarct volume was measured by TTC stain, together with hemorrhagic volume quantified by a spectrophotometric assay after 24 h of thrombolysis. The results show that rhTNK-tPA 1.6 mg/kg significantly improved the NDS after cerebral thromboembolism in rats at different TTW. RhTNK-tPA improved the NDS by 36.4% ($P < 0.05$), 41.7% ($P < 0.01$), 37.5% ($P < 0.01$), 36.0% ($P < 0.05$), and 26.1% ($P > 0.05$) at 1, 2, 3, 4.5, and 6 h TTW with 6 h treatment, respectively, and by 45.8% ($P < 0.01$), 50.0% ($P < 0.01$), 48.0% ($P < 0.05$), 37.5% ($P > 0.05$), and 28.0% ($P > 0.05$)at 1, 2, 3, 4.5, and 6 h TTW with 24 h treatment, respectively. Rt-PA improved the NDS by 40.9% ($P < 0.05$), 37.5% ($P < 0.05$), 33.3% ($P < 0.05$), 28.0% ($P > 0.05$), and 8.7% ($P > 0.05$) at 1, 2, 3, and 4.5 h TTW with 6 h treatment, respectively, and by 50.0% ($P < 0.01$), 50.0% ($P < 0.01$), 48.0% ($P < 0.05$), 33.3% ($P > 0.05$), and 12.0% ($P > 0.05$) at 1, 2, 3, 4.5, and 6 h TTW with 24 h treatment, respectively. RhTNK-tPA significantly reduced the extent of brain lesions examined by MRI at different TTW. At 1, 2, 3, 4.5, and 6 h TTW, lesion volume was reduced by 73.3% ($P < 0.001$), 64.2% ($P < 0.01$), 62.1% ($P < 0.01$), 46.1% ($P < 0.05$), and 39.6% ($P > 0.05$) at rhTNK-tPA dose of 1.6 mg/kg, respectively, and by 61.7% ($P < 0.01$), 59.4% ($P < 0.05$), 48.1% ($P < 0.05$), 49.1% ($P < 0.05$), and 17.8% ($P > 0.05$) at rt-PA dose of 9 mg/kg, respectively. RhTNK-tPA significantly reduced the extent of cerebral infarction examined by TTC at different TTW. At 1, 2, 3, 4.5, and 6 h TTW, infarction volume was reduced by 63.8% ($P < 0.01$), 71.0% ($P < 0.05$), 63.2% ($P < 0.05$), 58.0% ($P < 0.01$), and 35.7% ($P > 0.05$) at rhTNK-tPA dose of 1.6 mg/kg, respectively, and by 53.4% ($P < 0.01$), 60.9% ($P < 0.05$), 51.7% ($P < 0.05$), 54.6% ($P < 0.05$), and 20.4% ($P > 0.05$) at rt-PA dose of 9 mg/kg, respectively. The amount of hemorrhage in rhTNK-tPA rats increased slightly with the prolongation of the TTW ($P > 0.05$), and the amount of bleeding at 6 h TTW increased approximately 1.6 folds compared with the model group ($P = 0.0748$). The amount of hemorrhage in rt-PA rats increased with the prolongation of the TTW, and the amount of bleeding at 6 h TTW increased more than two folds compared with the model group ($P < 0.01$). Thus, rhTNK-tPA had an obvious therapeutic effect on ischemic stroke caused by thrombosis, and could be started within 4.5 h TTW.

31. Role of glutamate in the pathogenesis of acquired epilepsy in alzheimer's disease

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Alzheimer's disease (AD) can increase the risk of epileptogenesis up to 10-fold in the patient, compared to healthy age-matched controls. However, the relationship between acquired epilepsy and AD is yet to

be elucidated. Here we proposed that changes in the brain that occur early in the AD pathology may lead to this higher susceptibility to epileptogenesis. Disruption in brain's glutamate homeostasis has been reported in both disease and therefore it has the potential to link the two diseases together. This study aimed to explore the potential role of glutamate in the pathogenesis of acquired epilepsy in AD. It also aimed to identify potential early biomarkers or a diagnostic tool for acquired epilepsy in AD. Six month-old Tg2576 AD mice along with their wild-type (WT) littermate were utilised in this study. The cortex and the hippocampus were extracted from the animal, then western blotting and mass spectrometry were performed. Tg2576 had significantly lower amounts of GLT-1 and Glutamine synthetase in the cortex, compared to the WT. Additionally, mass spectrometry have shown that metabolites such as glutamate and glutamine have the potential to be the early biomarker for acquired epilepsy in AD. The results suggest that the astrocytic function could be impaired early in AD and this include the glutamate-glutamine cycle. This impairment might lead to a higher susceptibility of the brain to epileptogenesis via the excessive extracellular glutamate. The findings from the metabolomics analysis also suggest that there are changes in different brain's metabolites early in AD.

32. Late preterm infants' social competence, motor development, and cognition

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A preterm birth with a GA of 34 weeks 0 days to 36 weeks 6 days is called a late preterm birth; 70% of preterm births fall into this gestation period. Until a few years ago, late preterm birth was considered of no importance in the regular monitoring of babies' health, neurodevelopment, and social development. The aim of this study was to compare the social competence, motor development, and cognition of late preterm infants (LPIs) with full-term infants. Several studies in the recent past indicated that LPIs are at high risk of social development problems. We compared the development of motor skills, cognition, and social competency of LPIs with full-term infants at between 2 and 2.5 years old. The Chinese versions of the Gesell development diagnosis scale and the normal development of social skills from infants to junior high school children scale were used for the assessment. LPIs were not more socially competent than their full-term counterparts. Each skill, namely adaptability, gross motor, fine motor, language, and personal-social responses, was separately associated with the total level of social skills. It was found that gross motor skills had a positive correlation with the self-help and locomotive abilities, and fine motor skills had a positive association with locomotion abilities. LPIs had risk factors due to their delayed social skills in areas including motor disorders and physiological and perinatal factors. LPIs under three were at a higher risk of impairment in social competency. Therefore, it is recommended that they be monitored regularly to identify the development of social and cognitive disorders at an early stage.

33. The interaction of N-terminal motif of acetylcholinesterase and β-amyloid triggers β-amyloid aggregation and deposition

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Acetylcholinesterase (AChE) is one of the molecular chaperones inducing β-amyloid (Aβ) aggregation and deposition in the pathological process of Alzheimer's disease (AD). Peripheral anionic site (PAS) of AChE is

a binding site of Aβ with AChE. Our previous study found for the first time that the 7-20 amino acid region on the N-terminal of AChE (AChE7-20) can induce the formation of Aβ oligomer. However, the binding mode and pathophysiological effects of AChE7-20 and Aβ interaction remains unclear. In this study, protein-protein docking and molecular dynamics simulation were used to probe the interaction between AChE7-20 and Aβ. The residues of Arg13, Glu7, and Arg18 on AChE7-20 were the main contacts of AChE7-20 with Aβ. His13/14, Glu22/Asp23, and Asn27 on α-helix Aβ and Glu11, Phe19/20, Glu22/Asp23, and Met35 on β-sheet Aβ were the key residues of Aβ binding with AChE7-20, respectively. Compared with Aβ alone, AChE7-20-Aβ interaction triggered the transformation of Aβ from α helix to β sheet. The residues of Aβ participating in aggregation became fluctuant due to the presence of AChE7-20. The TEM morphology of Aβ aggregation confirmed that AChE7-20 induced more Aβ fibrils than control AChE7-20 in scramble sequence (Sc-AChE7-20). The binding affinity of AChE7-20 with Aβ oligomer was higher than Sc-AChE7-20 determined by SPR assay. Compared with Aβ alone, AChE7-20 co-incubation enhanced the apoptosis of primary hippocampal neurons induced by Aβ. Intervention of AChE-Aβ interaction was further studied with Bis-(9)-(-)-Meptazinol (BisMep), a novel dual-binding AChE inhibitor developed by our group. Results of molecular docking suggested that BisMep could not only interact with Tyr341 and Asp74 residues in the PAS of AChE through hydrogen bond and ionic bond, but also Arg13 and Arg16 residues in AChE7-20 through H-π and cation-π interaction, which are the key residues in AChE7-20-Aβ interaction. Subsequently, in Aβ-induced AD model mice, BisMep could significantly decrease the Aβ deposits, the activation of astrocytes and microglia, the levels of pro-inflammatory factors, and the AChE catalytic activity in the hippocampus, and eventually improve the performance of learning and memory of AD model mice. In conclusion, this study revealed the key residues and binding mode between AChE7-20 and Aβ and confirmed the triggering effect of AChE7-20 on Aβ aggregation and neurotoxicity, which provided structural biological information for the discovery of a lead compound intervening AChE-Aβ interaction.

34. Perceptual closure analysis: preliminary study with event-related potentials

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The perceptual closure is a complex visual skill that allows perceiving an incomplete pattern or object as complete or whole. A better knowledge of the electrophysiological bases of the perceptual closure would be useful to better understand the visuospatial alterations that occur in pathologies such as Williams syndrome, schizophrenia, and autism. Event-related potentials (ERP) studies in tasks with fragmented objects or faces have evidenced the presence of a component called Closure Negativity.

The objective of this study was to identify differences in ERP obtained from correctly or incorrectly fragmented figures in a perceptual closure task. Participants were 12 healthy male adults, university students between 20 and 31 years of age, who performed a visual closure task in which they had to decide if an incomplete figure corresponded (congruent condition) or not (incongruent condition) to a complete figure that was presented before. ERPs were recorded in 32 electrodes with a NeuroScan equipment. The ERP showed a negative peak around 170 ms (N1) without differences between the conditions, and a positive peak between 240 and 270 ms (P2) with greater amplitude for the incongruent condition than for the congruent one. Additionally, a negativity was observed around 400 ms that also had a greater amplitude for the incongruent *vs*. congruent condition. These preliminary findings give evidence of a differentiated processing for figures perceived as congruent *vs*. incongruent from a pattern, in latencies that could be related to late visual processing (P2) and probably to a perceptual semantic evaluation. This pattern should be confirmed with more studies, to be applied later in clinical populations such as those mentioned.