AO-01-1 Aberrant CHCHD2-Associated Mitochondriopathy in Kii ALS/PDC Astrocytes

○Satoru Morimoto[1,2,3], Nicolas Leventoux[1], Mitsuru Ishikawa[1], Shiho Nakamura[1], Fumiko Ozawa[1], Reona Kobayashi[1], Hirotaka Watanabe[1], Sopak Supakul[1], Satoshi Okamoto[1], Zhi Zhou[1], Hiroya Kobayashi[1], Chris Kato[1], Yoshifumi Hirokawa[2], Ikuko Aiba[1], Shinichi Takahashi[1,5], Shinsuke Shibata[1,6], Masaki Takao[7], Fumito Endo[8], Koji Yamanaka[8], Yasumasa Kokubo[9], Hideyuki Okano[1,3]

[1] Department of Physiology, Keio University, School of Medicine, Japan, [2] Department of Oncologic Pathology, Mie University Graduate School of Medicine, Mie, Japan, [3] Neurodegenerative disease research, Tokyo Metropolitan Institute for Geriatrics and Gerontology, Japan, [4] Department of Neurology, NHO Higashinagoya National Hospital, [5] Department of Neurology and Stroke, Saitama medical University, International Medical Centre, [6] Division of Microscopic Anatomy, Graduate School of Medical and Dental Sciences, Niigata University, [7] Department of Clinical Laboratory, National Centre of Neurology and Psychiatry (NCNP), [8] Department of Neuroscience and Pathobiology, Research Institute of Environmental Medicine, [9] Kii ALS/PDC Research Centre, Mie University Graduate School of Regional Innovation Studies

[Objective] Amyotrophic Lateral Sclerosis/Parkinsonism-Dementia Complex (ALS/PDC) is a rare and complex neurological disorder primarily found in the Western Pacific islands, such as Japan, Guam, and Papua-Neuguiniea. The root cause of the disease remains elusive, and genetic investigations have yet to provide definitive answers. Recent evidence suggests the potential involvement of astrocytes, crucial supporting cells in the brain, in the development and progression of Kii ALS/PDC. [Methods] To explore this further, our research team harnessed advanced induced pluripotent stem cell (iPSC) technology to cultivate multiple lines (Kii ALS/PDC 5 cases, Healthy 2 cases) of astrocytes for investigation. [Results] Among the findings, *CHCHD2* emerged as a significantly dysregulated gene when comparing disease astrocytes to healthy controls. Our analyses also uncovered imbalances in specific pathways associated with astrocytic cilium dysfunction, known to play a role in neurodegeneration, as well as pathways related to major neurological disorders, including classical ALS and Parkinson disease. More in-depth examinations revealed abnormalities in mitochondrial morphology and metabolic processes in affected astrocytes. A notable discovery was the reduced expression of CHCHD2 in the spinal cord of a patient with Kii ALS/PDC. [Conclusions] Our findings revelaed that astrocytes of Kii ALS/PDC provide reduced support to neurons, highlighting the potential role of CHCHD2 in maintaining mitochondrial health and its implications for the disease.

AO-01-2 Glia-neuron transmission of alpha-synuclein oligomers in an aggressive multiple system atrophy model

○Masaya Harada[1,2], Katsuhisa Masaki[1], Hiroaki Sekiya[3], Dai Matsuse[1], Hiroo Yamaguchi[1,4], Yuji Nishimura[1], Ezgi Ozdemir Takase[1], Eizo Tanaka[1], Tatsunori Tanaka[1,5], Yuu-ichi Kira[1], Dennis W. Dickson[3], Kei Fujishima[1], Eriko Matsuo[1], Ryo Yamasaki[1], Hideyuki Takeuchi[6,7,8], Takayuki Taniwaki[2], Noriko Isobe[1], Jun-ichi Kira[1,9,10]

[1] Department of Neurology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University, Japan, [2] Division of Respirology, Neurology and Rheumatology, Department of Medicine, Kurume University School of Medicine, Japan, [3] Department of Neuroscience, Mayo Clinic, Jacksonville, Florida, [4] School of Physical Therapy, Faculty of Rehabilitation, Reiwa Health Sciences University, [5] Sumitomo Pharma Co., Ltd., Osaka, [6] Department of Neurology, Graduate School of Medicine, International University of Health and Welfare, [7] Department of Neurology, International University of Health and Welfare Atami Hospital, [8] Department of Neurology and Stroke Medicine, Yokohama City University Graduate School of Medicine, [9] Translational Neuroscience Research Center, Graduate School of Medicine, and School of Pharmacy at Fukuoka, International University of Health and Welfare, [10] Department of Neurology, Brain and Nerve Center, Fukuoka Central Hospital

Aim: To assess α-synuclein (αSyn) oligomer formation in our new model of multiple system atrophy cerebellar type (MSA-C). **Methods:** Plp1-tTA::tetO- SNCA*A53T double transgenic (Tg) mice were generated to express mutant human A53T αSyn in oligodendroglia from 8 weeks of age. αSyn oligomers were studied by proximity ligation assay. Effects of a pan-connexin (Cx) blocker, INI-0602, on MSA-C and oligomers were clinico-histologically assessed using mice injected with INI-0602 or vehicle (n=16 each) from 18 weeks. **Results:** Tg mice developed rapidly progressive ataxia at 22 weeks and died by 30 weeks. They had prominent demyelination and glial inflammation in the brainstem. Brainstem oligodendroglia-like cells first multifocally harbored αSyn oligomers at 16 weeks in Tg but not wild-type mice. At 24 weeks, oligomer staining was slightly decreased in glia but some brainstem neurons showed punctate staining. At 30 weeks, oligomer staining was clustered mainly in the remaining brainstem neurons and occasionally in Purkinje cells. Phosphorylated αSyn (pαSyn) began to focally accumulate in the brainstem at 16 weeks, which was less than oligomers, and more extensively deposited at 24 and 30 weeks successively. INI-0602 significantly attenuated motor deterioration and glial inflammation while facilitated oligomer spreading but reduced pαSyn deposits. **Conclusion:** Our MSA-C model is the first to show glia-neuron transmission of αSyn oligomers. αSyn oligomers deposit preceding to pαSyn. INI-0602 facilitates oligomer propagation but blocks pαSyn formation from oligomers and attenuates MSA-C.

AO-01-3 Brain-resident CD8 T cells regulate inflammation in the early pathogenesis of Alzheimer's disease

○Asato Tsuji, Norio Chihara, Ritsu Akatani, Kimitaka Katanazaka, Shota Nishii, Riki Matsumoto

Division of Neurology, Kobe University Graduate School of Medicine, Japan

Objective:The factors that regulate inflammation in the early stages of Alzheimer's disease (AD) are not yet understood. We aimed to elucidate the role of T cells in the brain during the very early stages of AD. Methods:We used mutatant Aβ precursor protein (APP^{NL-G-F})KI mice (APP mice). Immune cells isolated from the cerebrospinal cord and other peripheral organs were stained with fluorescent antibodies and analyzed using flow cytometry. Frozen sections of brain were evaluated by immunofluorescence staining. In the T cell depletion experiment, intraperitoneal antibody administration was performed intermittently, and changes in immune cells were analyzed. Statistical analysis was performed with GraphPad Prism 9. Results:Brain T cells increased in an age-dependent manner, which was more pronounced for CD8 T cells. APP mice showed an earlier and greater increase in brain CD8 T cells than wild-type mice, (*p < 0.05, Mann Whitney test). CD8 T cells in the brain of APP mice expressed higher levels of PD-1, CD69, CD103, compared to other tissue (****p <0.0001, ordinary one-way) and had the characteristics of tissue-resident memory T cells (TRM). We then performed CD8 T cells depletion experiments in APP mice, and found an increase in the frequency of microglia compared to the control group. Conclusion: In APP mice, an increase in brain CD8 T cells characteristic of TRM and an increase in brain microglia due to CD8 T cell elimination therapy were observed. It is suggested endogenous brain CD8 T cells may regulate inflammation of brain microglia in the early stages of the disease.

AO-01-4 WIPI4 depletion associated with BPAN causes PE-dependent ferroptosis

○Motoki Fujimaki[1], Ye Zhu[2], Ana Lopez[2], David Rubinsztein[2], Shinji Saiki[1]

[1] Department of Neurology, University of Tsukuba, Japan, [2] Cambridge Institute for Medical Research, University of Cambridge

[Purpose]Beta-propeller protein-associated neurodegeneration (BPAN) is a rare X-linked dominant disease, categolized in neurodegeneration with brain iron accumulation. The gene that is mutated with loss-of-function in BPAN is *WDR45*, encoding WIPI4; however, the cellular mechanism by which the mutation triggers BPAN is unknown. Therefore, this study aimed to delineate the mechanisms underlying BPAN by creating genetically mimicked neuronal cell cultures and zebrafish models. [Methods] *WDR45* was silenced by siRNA knockdown or CRISPR knockout in SH-SY5Y neuroblastoma cells, shRNA knockdown in iPS cell-derived neurons and mouse primary neurons, and CRISPR knockdown in zebrafish. The induction of cell cytotoxicity and its mechanism were investigated in those models with more than three experiments for the proper statistical analysis. [Results] WIPI4 depletion caused ferroptosis, a type of cell death induced by lipid peroxidation. WIPI4 depletion also increased the localization of ATG2A, a lipid transfer protein interacting with WIPI4, at ER-mitochondria contact sites, which enhanced phosphatidylserine import into mitochondria. This resulted in increased mitochondrial synthesis of phosphatidylethanolamine, a major lipid prone to peroxidation, thus enabling ferroptosis. [Conclusion] Ferroptosis is one of the key mechanisms of cell cytotoxicity in BPAN neuronal models. This novel finding provides insights into the causes of neurodegeneration in BPAN, and may offer clues to therapeutic strategies. (This study is currently under revision for publication in Nature Cell Biology.)

AO-01-5 A cyclic pyrrole-imidazole polyamide: therapeutic agents for CAG/CTG triplet repeat diseases

○Susumu Ikenoshita[1,2], Kazuya Matsuo[1], Yasushi Yabuki[1], Sefan Asamitsu[1], Toshikazu Bando[3], Hiroshi Sugiyama[3,4], Mitsuharu Ueda[2], Norifumi Shioda[1]

[1] Department of Genomic Neurology, Institute of Molecular Embryology and Genetics, Kumamoto University, Japan, [2] Department of Neurology, Graduate School of Medical Sciences, Kumamoto University, Japan, [3] Department of Chemistry, Graduate School of Science, Kyoto University, [4] Institute for Integrated Cell-Material Science, Kyoto University

[Objective]Expansion of CAG and CTG (CWG) triplet repeats causes several inherited neurological diseases, including myotonic dystrophy type-1 (DM1) and Huntington's disease (HD). However, no effective therapeutic approach has been established. Then, we assessed the potential of a CWG triplet repeat DNA-targeting compound cyclic pyrrole-imidazole polyamide (CWG-cPIP) in inhibiting expanded CWG repeat-derived mRNA transcription, ameliorating neuronal dysfunction in CWG triplet repeat disease models. [Methods]To confirm the inhibitory effect of CWG-cPIP on transcription elongation by RNA polymerase, we performed an in vitro transcription arrest assay. Furthermore, we investigated the inhibitory effect of CWG-cPIP in cells from patients with DM1 and HD. To assess whether CWG-cPIP ameliorates in CWG repeat diseases in vivo, we evaluated in behavioral and immunohistochemical analysis in adeno-associated virus (AAV)-mediated CWG repeat-expressing mice and a genetic mouse model of HD. [Result]We found that CWG-cPIP selectively inhibits pathogenic mRNA transcripts from expanded CWG repeats, reducing RNA foci and polyQ accumulation in cells from patients with DM1 and HD. Treatment with CWG-cPIP ameliorated neuronal dysfunction in AAV-mediated CWG repeat-expressing mice and a genetic mouse model of HD. [Conclusions]CWG-cPIP exhibited high binding capacity for the CWG repeat DNA sequence, and its administration significantly restored the molecular, physiological, and behavioral impairment associated with CWG repeat diseases.

AO-01-6 Ecological and functional characteristics of the gut phageome in patients with multiple sclerosis

○Daiki Takewaki[1,2,3], Yuya Kiguchi[3,4], Hiroaki Masuoka[3], Wakiro Sato[1,2], Yukio Kimura[2,6], Noriko Sato[6], Yumiko Nakanishi[5], Hiroshi Ohno[5], Wataru Suda[3], Takashi Yamamura[1,2]

[1] Department of Immunology, National Center of Neurology and Psychiatry, Japan, [2] Multiple Sclerosis Center, National Center of Neurology and Psychiatry, Japan, [3] Laboratory for Symbiotic Microbiome Sciences, RIKEN, Japan, [4] Department of Computational Biology and Medical Sciences, Graduate School of Frontier Sciences, The University of Tokyo, [5] Laboratory for Intestinal Ecosystem, RIKEN, Center for Integrative Medical Sciences, [6] Department of Radiology, National Center of Neurology and Psychiatry

[**Objective**] Bacteriophage is one of the major components in the human gut microbiome, but the whole picture of the gut bacteriophage community (phageome) and its impact on human diseases remains largely unknown. The purpose of this research is to characterize the gut phageome in patients with multiple sclerosis (MS). [**Methods**] By applying state-of-the-art sequencing technologies combining short-read and long-read metagenomics, we constructed 6,185 high-quality human gut phage genomes from fecal samples derived from healthy control participants (HC; n=30), patients with relapsing-remitting MS (RRMS; n=40), and secondary progressive MS (SPMS; n=19). By using this phage catalog, we analyzed the precise composition and functions of the gut phageome, followed by the fecal metabolome analysis. [**Results**] Each patient group had a number of taxa having significant changes in abundance in comparison with HC, including Faecalibacterium infecting phages. Metagenomic functional analysis disclosed a significant reduction in phage-derived genes involved in coenzyme B12 biosynthesis, which was significantly correlated with an EDSS score and total brain volume of the patients. Moreover, we obtained the evidence indicating that the marked reduction of propionate, a representative disease-modifying metabolite in the gut of patients with MS was presumably regulated by coenzyme B12 biosynthesis. [**Conclusions**] We revealed ecological and functional characteristics of the gut phageome in patients with MS, which is possibly associated with the pathogenesis of this disease.

※AO-01 is the session of 65th JSN meeting / AOCN2024 program

APe-01-7 Combining salivary a-synuclein and miRNA-29a to distinguish multiple system atrophy and PD

○Mingyue Luan[1], Luhua Wei[1], Yunchuang Sun[1], Jing Chen[1], Yanyan Jiang[1], Fan Li[1], Wei Sun[1], Li Zhu[2], Zhaoxia Wang[1], Jianwen Deng[1]
[1] Peking University First Hospital, China, [2] Institute of Biophysics, Chinese Academy of Sciences

Introduction: The use of saliva biomarkers of neurodegeneration can diagnose patients with Lewy body disease and multiple system atrophy (MSA). The differential diagnosis of Parkinson's disease (PD) by single non-invasive salivary biomarkers is still a challenging task. **Objective:** to assess the diagnostic ability of two-step diagnostic approach that combined of salivary microRNA-29a-3p (miR-29a-3p) and RT-QuIC of a-synuclein (a-syn) in discriminating PD from atypical parkinsonisms (APDs). **Methods:** We included 208 patients from the Hospital with PD (n= 101), MSA (n=32),essential tremor (ET, n=22) and healthy control subjects (HCs, n=53). MiR-29a-3p in saliva was measured by real time quantitative PCR (RT-qPCR) as well as *α*-syn in saliva by RT-QuIC. **Results:** Sensitivity for PD was 70.30%, and specificity for healthy controls was 92.45%. Sensitivity for MSA was 56.25%. The expression level of saliva miR-29a-3p was significantly decreased in patients with PD ($p<0.001$) and MSA ($p<0.0001$), and allowed differentiation with HCs (PDvsHCs, AUC 0.69; MSAvsHCs, AUC0.91 PDvsMSA0.67.). The expression level of saliva miR-29a-3p in patients with ET was similar to HCs ($p>0.05$). The RT-QuIC to differentiate PD from MSA was difficult (AUC0.55), while the lag phase provided a good diagnostic value (AUC0.71). RT-QuIC assay yielded high diagnostic accuracy for HCsvsPD (AUC0.88) but lower diagnostic accuracy for HCsvsMSA (AUC0.76). **Conclusions:** We show the combined potential of saliva miR-29a-3p and saliva *α*-syn to differentiate PD from other neurodegenerative disorders.