AO-01-1 A new antisense oligos targeting alpha-synuclein improves motor function in Parkinson's model mouse

○Takuya Uehara[1], Chi-jing Choong[1], Masayuki Nakamori[1], Kumiko Nishiyama[1], Hideki Hayakawa[1], Yuuya Kasahara[2,3], Kousuke Baba[1], Tetsuya Nagata[4], Takanori Yokota[4], Hiroshi Tsuda[1], Satoshi Obika[2,3], Hideki Mochizuki[1]
[1] Graduate School of Medicine, Osaka University, Department of Neurology, [2] National Institutes of Biomedical Innovation, Health and Nutrition, [3] Graduate School of Pharmaceutical Sciences Osaka University, Department of Bioorganic Chemistry, [4] Graduate School Tokyo Medical and Dental University, Department of Neurology and Neurological Science

[Objective] A familial form of Parkinson's disease (PD) caused by the multiplication of Alpha-synuclein (SNCA) gene indicates that increasing levels of SNCA protein is a significant risk of PD. Importantly, accumulation of SNCA protein in the CNS neurons is a major pathology of sporadic PD, suggesting that reducing expression levels of SNCA might modify the course of the disease. Our aim is to establish antisense oligonucleotide (ASO) targeting SNCA gene for a novel therapy of PD effectively. [Methods] We designed and synthesized amido-bridge nucleic acids (AmNA)-modified ASO targeting against SNCA with improved stability and cellular uptake. We injected AmNA-ASO into intraventricular space of mice and examined its distribution in the brain. To examine the potency of AmNA-ASO, we measured levels of SNCA protein in the brain of transgenic mice expressing wild-type SNCA (Thy1-SNCA mice) by ELISA. To determine effects of AmNA-ASO on the behaviors, we performed wire suspending test and pasta gnawing test of the mice. [Results] AmNA-ASO could be efficiently delivered into adult mouse brain by intracerebroventricular injection without additional chemicals. AmNA-ASO significantly decreased the levels of SNCA protein expressing in the brain. Importantly, AmNA-ASO could ameliorate neurological defects observed in the Thy1-SNCA mice. [Conclusion] We have established AmNA-ASO targeting for SNCA efficiently. AmNA-ASO is a promising therapeutic strategy for PD patients. AmNA-ASOS will have broad implications for Alzheimer's disease, ALS, and other neurodegenerative diseases.

AO-01-2 a new devise HANABI facilitates diagnosis of Synucleinopaties by sonication induced amplification

○Kensuke Ikenaka[1], Keita Kakuda[1], Katsuya Araki[1], Masatomo So[2], Kentaro Noi[5], Shinichi Hashimoto[4], Hirotsugu Ogi[5], Hiroshi Tsuda[1], Takahiko Tokuda[3], Yuji Goto[2], Hideki Mochizuki[1]
[1] Department of Neurology, Osaka University Graduate School of Medicine, [2] Laboratory of Protein Folding, Institute of Peotein Research, Osaka University, [3] Department of Neurology, Kyoto Prefectural University of Medicine, [4] Corona Electrical Co., [5] Department of Mechanical Science and Bioengineering, Osaka University Graduate School of Engineering Science

[Objectives] Parkinson's disease (PD) and multiple systemic atrophy (MSA) share a common pathology caused by abnormal accumulation of Alfa-Synuclein (SNCA) in the brain. It remains to be understood why the misfolded SNCA causes distinct clinical phenotypes. We have recently developed a new devise HANABI based on Real-Time Quaking-Induced Conversion (RT-QuIC) analysis. HANABI can amplify and detect misfolded SNCA in the CSF in a very sensitive and specific manner. Our aim is to determine structure variations of SNCA accumulated in PD and MSA patients using HANABI. [Methods] We performed RT-QuIC analysis of CSF samples obtained from 40 PD, 7 MSA and 20 control. To examine structure variations of SNCA, we analyzed and monitored kinetics of fibril amplification by HANABI. To determine correlation of the SNCA aggregation and disease progression, we compared kinetics with clinical scores and imaging data. To examine detailed structures of amplified SNCA fibril, we also performed several biochemical and ultrastructural analyses, including proteinase K treatment, FT-IR, and TEM. [Results and Conclusions] The kinetics of PD and MSA patients were significantly faster than that of control. Interestingly, TEM and FT-IR analysis revealed that SNCA fibril amplified from PD and MSA were distinct, suggesting the structural differences between two fibrils. Our data suggest that structure variations of SNCA causes distinct pathology of PD and MSA. HANABI will be useful for facilitating diagnosis of PD and MSA and allow us to understand a novel mechanisms of disease associated with SNCA.

AO-01-3 Successful treatment of hypertrophic pachymeningitis by transforming growth factor-beta blockade

○Yiwen Cui[1], Katsuhisa Masaki[1], Ryo Yamasaki[1], Shotaro Hayashida[1], Hiroo Yamaguchi[1], Fuminori Hyodo[2], Hinako Eto[2], Tomomi Yonekawa[1], Takuya Matsushita[1], Mari Yoshida[3], Marie Malissen[4], Bernard Malissen[4], Jun-ichi Kira[1]
[1] Department of Neurology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University, [2] Innovation Center for Medical Redox Navigation, Kyushu University, [3] Department of Neuropathology, Institute for Medical Science of Aging, Aichi Medical University, [4] Centre d'Immunologie de Marseille-Luminy, Aix Marseille Université, INSERM, CNRS

Purpose: Hypertrophic pachymeningitis (HP) lacks an animal model to elucidate its underlying pathomechanisms. We reported that IgG4-related disease is a major cause of HP by nationwide survey. Mice with a mutation (Y136F) in linker for activation of T cells (LAT) result in IgG1 (IgG4 human equivalent) overexpression. We determined whether LAT mutant mice could be used as a model of IgG4-related HP. **Methods:** We used MRI, neuropathological method and western blotting to longitudinally evaluate dura in mice of LAT mutant mice at aged 3, 6 and 13 weeks. We also evaluated autopsied dura specimens from patients with HP. **Results:** Dural gadolinium enhancement began focally around the superior sagittal sinus in 3-week-old LAT mutant mice, extending over the brain in the ensuing 13 weeks. Dural lesions showed massive infiltration of plasma cells, B, T, and IgG1-positive cells, macrophages, and neutrophils at 3 weeks, followed by marked fibrotic thickening. TGF-β1 immunoreactivity was markedly upregulated and SMAD2/3 was phosphorylated in the dural lesions of both LAT mutant mice and human HP patients. Western blotting showed marked upregulation of TGF-β1 and phosphorylation of SMAD2/3 in dura of LAT mutant mice. Daily oral irbesartan administration, an angiotensin II receptor type I (AT1) blocker, starting at age 3 weeks nearly abolished dural inflammation and fibrotic thickening. **Conclusion:** Our findings demonstrate that LAT mutant mice can be a suitable model of IgG4-related HP. TGF-β and SMAD2/3 blockade by AT1 blocker may provide a novel therapeutic approach for intractable HP.

AO-01-4 ALS-associated C21ORF2 mutation enhances the autoregulation mechanism of NEK1

○Yasuaki Watanabe[1,2], Tadashi Nakagawa[1], Naoki Suzuki[2], Hitoshi Warita[2], Keiko Nakayama[1], Masashi Aoki[2]
[1] Division of Cell Proliferation, ART, Tohoku University Graduate School of Medicine, [2] Department of Neurology, Tohoku University Graduate School of Medicine

[Purpose] Two genes, *NEK1* and *C21orf2* were reported as novel ALS-associated genes in 2016. Intriguingly, NEK1 and C21orf2 interact with each other, and both of them are demonstrated to be mutated in some ciliopathies, suggesting they play a crucial role cooperatively in ALS patients. Our purpose was deciphering the interactive functions between C21ORF2 and NEK1, and the effect on them caused by V58L mutation of C21orf2 which is observed in ALS patients.[Methods] We investigated protein stability of C21orf2 as well as NEK1 in HEK293T cells. For functional analysis, we generated C21orf2-disrupted mIMCD3 cells. [Results] We found C21orf2 was a novel substrate of E3 ligase SCF^{FBXO3}. Poly-ubiquitylated C21orf2 was degraded by proteasome. NEK1 phosphorylated C21orf2, leaded to increase the C21orf2 protein by inhibiting its ubiquitylation. V58L mutant of C21orf2 is not ubiquitylated and was stable compared to wild type. On the other hand, we noticed C21orf2 stabilized NEK1 protein vice versa. In C21orf2-disrupted mIMCD3 cells, we observed extreme reduction of endogenous NEK1. The cyclohexamide chase assay revealed that overexpression of C21orf2 suppressed the degradation of NEK1. [Conclusion] We demonstrated that NEK1 regulates its protein amount by a positive autoregulation system in which NEK1 and C21orf2 stabilize each other. The amount of V58L-C21orf2 increases because of not ubiquitylation results in unnecessary stabilization of NEK1. In ALS patients who suffer the *C21orf2* rare coding variants, NEK1 overexpression is suggested to be one of the center of ALS pathogenesis.

AO-01-5 Developmental YAPdeltaC determines adult pathology in a mouse model of spinocerebellar ataxia type 1

○Kyota Fujita, Ying Mao, Shigenori Uchida, Xigui Chen, Hiroki Shiwaku, Takuya Tamura, Hikaru Ito, Hidenori Homma, Kazuhiko Tagawa, Hitoshi Okazawa
Department of Neuropathology, Medical Research Institute, Tokyo Medical and Dental University

[Objective] YAP and its neuronal isoform YAPdeltaC are implicated in various cellular functions including an atypical form of cell death named TRIAD (transcriptional repression-induced atypical cell death). Here we tested the therapeutic effects of YAPdeltaC on SCA1 pathology using a newly developed Tet-ON YAPdeltaC system in mutant Ataxin-1 knock-in (Atxn1-KI) mice. [Method] We crossed YAPdeltaC transgenic mice, which expression were increased from embryo (P0) or adult (8 weeks old) under the control of doxycycline, with mutant Atxn1-KI mice. We performed behavioral, morphological, and biochemical analysis to evaluate the effects to SCA1 pathology. [Result] The expression of YAPdeltaC during development, but not adulthood, rescued neurodegeneration phenotypes of mutant Atxn1-KI mice. We found that YAP/YAPdeltaC interacted with RORalpha and served as co-activators of its transcriptional activity. YAP/YAPdeltaC formed a transcriptional complex with RORalpha. Both normal and mutant Atxn1 interacted with YAP/YAPdeltaC, while only mutant Atxn1 depleted YAP/YAPdeltaC from RORalpha complex to suppress transcription on short timescales. Over longer periods, mutant Atxn1 also decreased RORalpha in vivo. Genetic supplementation of YAPdeltaC restored the level of RORalpha and YAP/YAPdeltaC, recovered YAP/YAPdeltaC in the RORalpha complex and normalized target gene transcription in Atxn1-Ki mice in vivo. [Conclusion] Functional impairment of YAP/YAPdeltaC by mutant Atxn1 during development determines the adult pathology in SCA1 by suppressing RORalpha-mediated transcription.

AO-01-6 The effect of exercise in a mouse model of spinal and bulbar muscular atrophy

○Hideaki Nakatsuji[1], Naohide Kondo[1], Madoka Iida[1], Genki Tohnai[1], Kentaro Sahashi[1], Masahisa Katsuno[1], Gen Sobue[2]
[1] Department of Neurology, Nagoya University Graduate School of Medicine, [2] Research Division of Dementia and Neurodegenerative Disease, Nagoya University Graduate School of Medicine

Objective: Spinal and bulbar muscular atrophy (SBMA) is a neuromuscular disease caused by the expansion of a CAG repeat in the androgen receptor (AR) gene. Several studies indicate that physical exercise is likely to be protective in neuromuscular diseases. It is also reported that excessive exercise is associated with risk of motor neuron disease. Whether physical exercise is beneficial or harmful is still under debate. We aimed to investigate the effect of exercise in SBMA model mice. **Methods:**To investigate the effect of physical training at different stages of the disease, exercise was started from the pre or post-onset stage (5 or 9 weeks of age) in a transgenic mouse model of SBMA (AR-97Q). Exercise was loaded by forced wheel running cages for 1 hour a day, 5 days a week, and maintained for 4 weeks. We then performed behavioral and biochemical analysis on these mice and compared the results with those of sedentary AR-97Q mice as control.**Results:** The exercise from the pre-onset stage improved behavioral and histopathological findings, and extends the life span of AR-97Q mice. By contrast, the motor function and life span of the AR-97Q mice that underwent exercise at the post-onset stage were similar to those of the sedentary AR-97Q mice. In biochemical analysis, we found that the exercise from the pre-onset stage inhibits mutant AR accumulation in the skeletal muscles of AR-97Q mice. **Conclusion:** The present study showed that the exercise from the pre-onset stage mitigated symptoms and increased life span of SBMA model mice via reducing AR accumulation in skeletal muscle.

AO-02-1 Fc fusion protein as a novel treatment for myasthenia gravis

○Akiyuki Uzawa[1], Masayuki Homma[2], Hitoshi Tanaka[2], Naoki Kawaguchi[1], Tetsuya Kanai[1], Keiichi Himuro[1], Fumiko Oda[1], Yukiko Ozawa[1], Manato Yasuda[1], Satoshi Kuwabara[1]
[1] Department of Neurology, Graduate School of Medicine, Chiba University, [2] Research Laboratory, Nihon Pharmaceutical Co. Ltd.

【目的】重症筋無力症（MG）は神経筋伝導が障害されることで易疲労性を伴う全身の筋力低下を呈し、患者の多くがアセチルコリン受容体（AChR）対する自己抗体を有する。ステロイドを中心とした既存治療では長期寛解例が少なく新規治療の開発が求められている。我々はAChRのα1サブユニットの細胞外ドメイン構造にヒトIgG1のFc部位を結合させた融合蛋白であるAChR-Fcを作成し、その作用を抗AChR抗体、抗AChR抗体産生B細胞及びMGの動物モデル（EAMG）を用いて検証することを目的とした。【方法】(1) 融合蛋白AChR-Fcと抗AChR抗体（ハイブリドーマ細胞由来、MG患者由来[n=16]）の結合試験を行い、融合蛋白の自己抗体中和活性を検討した。(2) 融合蛋白AChR-Fcの自己抗体産生B細胞への結合及び傷害活性をハイブリドーマ細胞、患者由来の抗AChR抗体産生B細胞を用いて検討した。(3) ラットを用いたEAMG（各群n=6-8）に融合蛋白AChR-Fcを投与し重症度への影響を検討した。【結果】融合蛋白AChR-Fcは抗AChR抗体と濃度依存的に結合し、デコイとして自己抗体中和活性を示した。さらに融合蛋白AChR-FcはB細胞受容体を介して病原性B細胞と結合し、Fc領域とエフェクター細胞による抗体依存性細胞傷害（ADCC）活性により、抗AChR抗体産生B細胞を傷害した。また、ラットEAMGに融合蛋白AChR-Fcを投与したところ濃度依存的にMG症状を緩和し、抗AChR抗体価の低下が確認された。【結論】融合蛋白AChR-Fcは（1）抗AChR抗体との結合作用による自己抗体中和活性（2）ADCC活性による病原性B細胞傷害という2つの作用機序を有しMGの新規治療として有望である。Fc融合技術は他の抗体介在性免疫疾患にも応用できる革新的治療法と考えられる。

AO-02-2 Availability of J-CAT for Nation-Wide Prospective Cohort Studies of Spinocerebellar Degeneration

○Shinji Oda, Yuji Saitoh, Shoko Watanabe, Yuji Takahashi, Hidehiro Mizusawa, Japan Consortium of Ataxias
Department of Neurology, National Center Hospital, National Center of Neurology and Psychiatry

[Objective]Spinocerebellar degeneration (SCD) is a group of heterogeneous disorders in which genetic analyses are necessary to define the molecular background. We have developed the J-CAT, a cloud-based nation-wide registry for SCD, to establish the molecular diagnosis and disease-specific prospective natural history. The purpose of our study was to elucidate the current status of J-CAT and to focus on the clinical characteristics of SCA31 patients, thereby assessing the availability of the system. [Methods]We extracted the registration status of J-CAT including the geographical data to assess its accessibility. Mutational analysis was conducted for triplet repeat diseases and SCA31. We evaluated the disease duration and the SARA scores of SCA31 patients as clinical variables.[Result]Until November 2017, 304 patients have been registered from the 135 hospitals/clinics across 41 prefectures. Among them, 129 patients were subjected to the initial genetic tests, in whom 19 patients were diagnosed as SCA31 (14.7%). The mean age (year-old) at the onset, registration, and disease duration (year) of SCA31 patients was 56.5, 65.4, and 8.9, respectively. The mean SARA scores at the registration was 13.4.[Conclusion]This study showed that the J-CAT facilitates the registration of SCD patients with genetically confirmed diagnosis nation-wide. Because majority of SCA31 patients registered in the J-CAT were in early stage, our cohort would be available for prospective studies. J-CAT provides useful platform to promote the disease-specific prospective natural history studies for SCD.

AO-02-3 Pitfalls in clinical diagnosis of anti-NMDA receptor encephalitis

○Atsushi Kaneko[1], Takahiro Iizuka[1], Juntaro Kaneko[1], Naomi Kanazawa[1], Hiroki Suga[1], Naomi Tominaga[1], Daisuke Ishima[1], Eiji Kitamura[1], Kasumi Hattori[2], Yoshikazu Ugawa[2], Daisuke Kuzume[3], Kazutoshi Nishiyama[1]
[1] Department of Neurology, Kitasato University School of Medicine, [2] Department of Neurology, School of Medicine, Fukushima Medical University, [3] Department of Neurology, Chikamori Hospital

Objectives: To report pitfalls in clinical diagnosis of anti-NMDA receptor encephalitis (NMDARE). **Methods:** We retrospectively reviewed the clinical information of 221 patients with clinically-suspected autoimmune neurological disorders who underwent testing for antibodies (abs) against neuronal cell surface antigens between January 2007 and September 2017. Forty-one patients met the diagnostic criteria for probable NMDARE (probable criteria), but one was excluded because neither serum nor CSF obtained at active stage was examined. In 220 patients, sensitivity, and specificity of the probable criteria were assessed. **Results:** NMDAR-abs were detected in 34 of 40 patients (85%) with the probable criteria; however, 2 of the 6 ab-negative patients had ovarian teratoma. The median age at onset was higher in ab-negative patients than those with abs (49 vs. 27 years, p=0.015) with a probability of antibody detection <50% in patients (>50 years). NMDAR-abs were detected in 5 of 180 patients who did not fulfill the probable criteria; these patients presented with isolated epileptic syndrome (n=2, 1 with MOG-abs), atypical demyelinating syndrome (n=2, 1 with AQP4-abs), and autoimmune post-herpes simplex encephalitis (Post-HSE) (n=1). Sensitivity and specificity was 87.2% (34/39) and 96.7% (175/181), respectively. **Conclusion:** The probable criteria are valid, but diversity of clinical phenotype should be taken into account in diagnosing NMDARE particularly in patients who were 50 years or older, or presented with isolated epileptic syndrome, atypical demyelinating syndrome, or post-HSE.

AO-02-4 Clinical features and antigenic epitopes in anti-plexin D1 antibody-associated neuropathic pain

○Takayuki Fujii[1], Ryo Yamasaki[1], Kyoko Iinuma[1], Daisuke Tsuchimoto[2], Mizuho Kido[3], Shinichi Aishima[4], Yusaku Nakabeppu[2], Jun-ichi Kira[1]
[1] Department of Neurology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan, [2] Department of Immunobiology and Neuroscience, Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan, [3] Department of Anatomy and Physiology, Faculty of Medicine, Saga University, Saga, Japan, [4] Department of Pathology and Microbiology, Faculty of Medicine, Saga University, Saga, Japan

Objective: We recently discovered that anti-small dorsal root ganglion (DRG) neuron autoantibodies in patients with neuropathic pain (NeP) specifically reacted with plexin D1. We investigated the clinical features and antigenic epitopes of anti-plexin D1 antibody-positive patients. **Methods:** Nine of 11 patients with anti-small DRG neuron antibody were confirmed to have anti-plexin D1 antibodies by cell-based small interfering RNA assay. We retrospectively reviewed patient medical records and performed a current perception threshold (CPT) test before and after immunotherapy. Mouse tissue-based indirect immunofluorescence assay (IFA) identified IgG antibody subclass and reactivity to carbohydrate epitopes by deglycosylation methods. **Results:** Anti-plexin D1 antibody-positive patients showed characteristic features including marked female preponderance, younger age at onset, burning and tingling pain with thermal hyperalgesia, peripheral vascular dysfunction symptoms and CPT abnormalities for C-fibers. Main comorbidities were atopy in 9 patients and collagen-vascular disease in 3. Immunotherapies ameliorated NeP and CPT abnormalities. Anti-plexin D1 IgG were mainly IgG2 and did not bind to DRG neurons after periodate oxidation which removes both N- and O-linked glycans, but after treatment with peptide-N-glycosidase F which removes N-linked glycan, and O-glycosidase which removes O-linked glycan but not O-mannose, anti-plexin D1 IgG still bound to DRG neurons. **Conclusions:** Anti-plexin D1 antibody carriers have unique NeP features and O-mannose glycans may be antigenic epitopes.

AO-02-5 Clinical features of autoimmune gastrointestinal dysmotility in Japan

○Akihiro Mukaino[1,2], Shunya Nakane[1,3], Hitomi Minami[4], Hajime Isomoto[5], Osamu Higuchi[6], Tohru Okanishi[7], Kenya Murata[8], Akio Ido[9], Eikichi Ihara[10], Yasuhiro Maeda[6], Hidenori Matsuo[6], Kazuhiko Nakao[4], Yukio Ando[1]
[1] Department of Neurology, Graduate School of Medical Sciences, Kumamoto University, [2] Department of Molecular Neurology and Therapeutics, Kumamoto University Hospital, [3] Department of NeAsian Neurological Intractable Diseases Research and Clinic, Graduate School of Medical Sciences, Kumamoto University, [4] Department of Gastroenterology and Hepatology, Nagasaki University Graduate School of Biomedical Sciences, [5] Division of Medicine and Clinical Science, Department of Multidisciplinary Internal Medicine, School of Medicine, Tottori University Faculty of Medicine, [6] Department of Clinical Research, Nagasaki Kawatana Medical Center, [7] Department of Child Neurology, Seirei Hamamatsu General Hospital, [8] Department of Neurology, Wakayama Medical University, [9] Digestive and Lifestyle Diseases, Department of Human and Environmental Sciences, Kagoshima University Graduate School of Medical and Dental Sciences, [10] Department of Medicine and Bioregulatory Science, Graduate School of Medical Sciences, Kyushu University

【背景】自己免疫機序による消化管運動障害としてautoimmune gastrointestinal dysmotility（AGID）は2008年に米国より提唱された疾患概念であり，食道・胃の運動障害や慢性偽性腸閉塞（CIPO）の一部を含有する．AGIDには抗自律神経節アセチルコリン受容体（gAChR）抗体などの様々な自己抗体が検出されることが報告されているが、自己免疫性自律神経節障害（AAG）の限局型であるのか、また抗gAChR抗体がその病態に関与しているのかは不明である.【目的】本邦におけるAGIDの臨床的特徴，治療反応性を明らかにする．【方法】1）抗gAChR抗体陽性AAG患者123症例におけるAGID（食道機能障害，胃不全麻痺，麻痺性イレウス）の頻度，臨床像，治療反応性を調査する．2）新たにアカラシア28症例，CIPO14症例における抗gAChR抗体陽性頻度を調査しその臨床像および自律神経症状の重症度スコアCOMPASS31を前向きに検討する．抗体測定にはルシフェラーゼ免疫沈降法を用いる．【結果】1）123症例のうち，食道機能障害6症例，胃不全麻痺2症例，麻痺性イレウス2症例を確認し，免疫治療による消化管運動機能改善症例を認めた．2）アカラシアでは6症例，CIPOでは7症例が抗体陽性で、自律神経障害（乾燥症状や膀胱機能障害など）の合併していた．COMPASS31において、抗gAChR抗体陽性CIPOでは排尿障害の頻度が有意に高かった.【結論】本邦の抗gAChR抗体陽性AAGにおいて重篤な消化管運動障害が存在し，免疫治療による有効性が示された．自律神経障害を併存していることからもAAGの限局型である可能性がある．神経内科医や消化器内科医は原因不明の消化管障害の病態に抗gAChR抗体の関与を考える必要がある．

AO-02-6 Serum caffeine and metabolites are reliable biomarkers of early Parkinson's disease

○Motoki Fujimaki[1], Shinji Saiki[1], Li Yuanzhe[1], Naoko Kaga[2], Hikari Taka[2], Taku Hatano[1], Kei-ichi Ishikawa[1], Yatake Oji[1], Akio Mori[1], Ayami Okuzumi[1], Takahiro Koinuma[1], Shin-ichi Ueno[1], Yoko Imamichi[1], Takashi Ueno[2], Yoshiki Miura[2], Manabu Funayama[1], Nobutaka Hattori[1]
[1] Juntendo University School of Medicine, Department of Neurology, [2] Juntendo University of Medicine, Laboratory of Proteomics and Biomolecular Science

[Background] PD is a neurodegenerative disease characterized by progressive dopaminergic neuronal loss especially in the substantia nigra. Epidemiologically, daily intake of caffeine reduces the onset risk of Parkinson's disease and *in vivo* experimental studies show that caffeine treatment attenuates neurotoxicity, however; the kinetics and metabolism of caffeine in human blood remains unclear. [Study purpose] In this study, caffeine and 10 caffeine-metabolized chemicals were assessed by liquid-chromatography mass-spectrometry. Also genetic variations in *CYP1A2* and *CYP2E1*, encoding cytochrome P450 relatively specific for caffeine metabolism. [Results] Caffeine and 9 downstream metabolites were significantly decreased even in serum of early PD patients unrelated to the amount of caffeine consumption or disease severity. No significant genetic variations of *CYP1A2* and *CYP2E1* were detected compared to the controls. Caffeine concentrations in PD with motor complications and more severity were significantly decreased, whilst no association with single nucleotide variations of *ADORA2A* gene encoding adenosine 2A receptor was detected. A set of caffeine and its downstream metabolites were identified as diagnostic biomarkers by receiver operating curve analysis.[Conclusion] These results suggest that a set of absolute lower levels of caffeine and its metabolites would be promising early diagnostic biomarkers for PD, consistent with a neuroprotective effect of caffeine approved by epidemiological and experimental studies.

AP-01-1 Brain-derived exosomes as potential blood biomarkers for Parkinson's disease and parkinsonism

○Takuma Ohmichi[1], Masato Mitsuhashi[2], Harutsugu Tatebe[1,3], Takashi Kasai[1], Toshiki Mizuno[1], Takahiko Tokuda[1,4]
[1]Department of Neurology, Kyoto Prefectural University of Medicine, [2]NanoSomiX, Inc., [3]Department of Zaitaku（Homecare）Medicine, Kyoto Prefectural University of Medicine, [4]Department of Molecular Pathobiology of Brain Diseases, Kyoto Prefectural University of Medicine

[Objective] There is still a substantial unmet need for blood-based biomarkers to make an objective diagnosis of Parkinson's disease (PD) and parkinsonism. The aim of this study is to determine whether enumeration of brain-derived exosomes in plasma is informative in the diagnosis of those diseases. [Methods] We have developed a novel method to enumerate the plasma levels of neuron-, astrocyte-, and oligodendrocyte-derived exosomes (NDEs, ADEs and ODEs, respectively) obtained from patients with PD, multiple system atrophy (MSA), progressive supranuclear palsy (PSP) and control subjects. [Results] The plasma levels of NDEs, ADEs, and ODEs were individually and precisely quantified with our novel assay system using antibodies against neuron-, astrocyte- or oligodendrocyte-specific proteins combined with an antibody to the exosome common marker CD81. The plasma levels of NDE, ADE and ODE were significantly higher in PD samples than in control samples, respectively (p=0.003 for NDE, p=0.021 for ADE, and p=0.008 for ODE). The plasma levels of ODE showed a significant correlation with UPDRS part III scores in the patients with MSA predominated in parkinsonism (MSA-P) (r^2=0.57, n=6, p=0.048) and those scores in the patients with PD (r^2=0.51, n=14, p=0.0041), respectively. [Conclusions] This is the first report that enumerates NDE, ADE, and ODE in human plasma and shows the potential usefulness of these levels as biomarkers for PD. Our results suggest the capability of the plasma levels of ODE as a surrogate biomarker to monitor the severity of parkinsonism in MSA-P and PD.

AP-01-2 Pathophysiological analysis of spinal and bulbar muscular atrophy using disease-specific iPSCs

○Kazunari Onodera[1,2], Takuji Ito[1], Daisuke Shimojo[1,3], Yasuharu Ishihara[3], Manabu Doyu[1], Masahisa Katsuno[2], Hideyuki Okano[3], Gen Sobue[4], Yohei Okada[1]
[1]Department of Neurology, Aichi Medical University School of Medicine, [2]Department of Neurology, Nagoya University Graduate School of Medicine, [3]Department of Physiology, Keio University School of Medicine, [4]Research Division of Dementia and Neurodegenerative Disease, Nagoya University Graduate School of Medicine

[Objective] Spinal and bulbar muscular atrophy (SBMA) is an adult onset neuromuscular degenerative disease caused by a CAG repeat expansion in androgen receptor (AR) gene. So far, the findings from mice models indicated that testosterone-dependent mutant AR aggregations play important roles in neuronal dysfunction and degeneration. We generated induced pluripotent stem cells (iPSCs) from SBMA patients to establish more accurate disease models, and investigated the pathogenesis of SBMA. **[Methods]** We established iPSCs from 4 SBMA patients' fibroblasts and 3 age-matched controls. For pathophysiological analysis of SBMA, iPSC-derived motor neurons (MNs) were cultured with dihydrotestosterone for 4 weeks. **[Results]** Mutant AR aggregations were not detected in 4-week SBMA-MN culture by immunocytochemistry or Western blot analysis, while these SBMA-MNs showed alteration of the expressions of several genes including *CALCA* (CGRP-1) and *TβR2* that are known to be associated with the early pathology of SBMA. By microarray analysis, several biomarker candidates were identified including *CALCA* (CGRP-1). For the analysis of advanced disease, we screened several small molecular compounds to find disease accelerating factors, and found that ER stress inducers, tunicamycin and thapsigargin, significantly enhanced the neurodegenerative phenotypes of SBMA-MNs. **[Conclusions]** SBMA-MNs recapitulated the early pathology of SBMA, and showed the vulnerability to ER stress. With this model, uncovering SBMA early pathology and identification of novel biomarkers and therapeutic targets are expected.

AP-01-3 alpha-synuclein propagation in brains via olfactory pathway in non-human primate model

○Masanori Sawamura, Norihito Uemura, Ryosuke Takahashi
Department of Neurology, Graduate school of Medicine, Kyoto University

Introduction: Parkinson's disease (PD) is the neurodegenerative disease characterized by aggregation of α-synuclein（α-syn）, called Lewy body. The α-syn aggregates are believed to propagate in brains in a prison-like fashion via two major pathways: the olfactory and vagal pathways. Recently, the common marmoset (*Callithrix jacchus*) has gathered a lot of attention in the field of neuroscience because of its useful characteristics as an animal model. In this study, we inoculated α-syn fibrils in the olfactory bulb of a common marmoset and analyzed its resulting pathology. Methods: Recombinant full-length marmoset α-syn was purified and incubated with agitation for a week to generate α-syn fibrils. A two-year-old female common marmoset was anesthetized with ketamine and isoflurane/oxygen mixture. Then, 0.8ul of fibrils solution (4mg/ml in sterile PBS) was stereotaxically injected using glass capillary at two sites in the unilateral olfactory bulb (OB). Three months after α-syn fibrils injection, the marmoset was sacrificed and perfused with PBS followed by 4% PFA in PBS. Eight-μm coronal sections were made and immunostained using phosphorylated α-syn (p-α-syn) antibody followed by DAB staining. Results: Widespread p-α-syn positive cells were observed in the ipsilateral OB, piriform cortex and amygdale. Importantly, we observed very few p-α-syn pathology in the contralateral side. We created a new non-human primate PD model triggered in olfactory bulb.

AP-01-4 A novel cell transplantation therapy for ALS using OPCs expressing scFv recognizing misfolded SOD1

○Sumio Minamiyama[1,2], Ryota Hikiami[1,2], Yoshitaka Tamaki[2], Akemi Shoudai[2], Takakuni Maki[1], Keizo Tomonaga[3], Ryosuke Takahasi[1], Makoto Urushitani[2]
[1]Department of Neurology, Kyoto University Graduate school of Medicine, [2]Department of Neurology, Shiga University of Medical Science, [3]Institute for Frontier Life and Medical Sciences, Kyoto University

[Objective] Mutations in superoxide dismutase1 (SOD1) are a leading cause of familial amyotrophic lateral sclerosis (ALS). Previous studies have demonstrated that the misfolded SOD1 could spread from cell-to-cell. Recently, the dysfunction and the accelerated turnover of oligodendrocyte and its precursor cell (OPC) have been implicated in the pathogenesis of ALS from the early stage. Therefore, we will test a combination therapy of OPC transplantation and antibody targeting extracellular SOD1 to improve aberrant oligodendrocyte environment and to prevent propagation of misfoled SOD1.[Methods]cDNA for tandem single chain of an antibody (named clone X) recognizing misfolded SOD1 specifically (scFv-X) was constructed, and was subcloned into a Borna disease virus (BDV) vector. Primary OPCs was infected by the BDV carrying scFv-X. We investigated whether the infected OPC can secrete scFv-X, and recognize misfolded SOD1. We also performed Immunohistochemical investigation of SOD1H46R-transgenic rat for OPCs.[Results]The mammalian expression vector expressing scFv-X derived from BDV was constructed and was subcloned into BDV vector. Primary OPCs infected by the BDV, secreted scFv-X, which recognize misfolded SOD1 specifically. OPC were remarkably proliferated in motor cortex and spinal cord of SOD1H46R rats.[Conclusions]OPC transplantation is a potential therapy due to its accelerated turnover. We succeeded in establishing a primary OPCs expressing misfolding-specific scFv. Further *in vivo* evaluation is expected to validate the effect of our combination therapy of OPCs and scFv.

AP-01-5 Novel binding partner of dysferlin is a potential therapeutic target for dysferlinopathy

○Hiroya Ono[1], Naoki Suzuki[1], Shin-ichiro Kanno[2], Genri Kawahara[3], Rumiko Izumi[1], Toshiaki Takahashi[1], Yasuo Kitajima[5], Shion Osana[6], Tetsuya Akiyama[1], Kensuke Ikeda[1], Tomomi Shijo[1], Shio Mitsuzawa[1], Hitoshi Warita[1], Ryoichi Nagatomi[6], Nobukazu Araki[7], Akira Yasui[2], Yukiko K. Hayashi[3], Katsuya Miyake[7,8], Masashi Aoki[1]
[1]Department of Neurology, Tohoku University School of Medicine, [2]The Institute of Development, Aging and Cancer, Tohoku University, [3]Department of Pathophysiology, Tokyo Medical University, [4]National Hospital Organization Sendai-Nishitaga National Hospital, [5]Department of Stem Cell Biology, Atomic Bomb Disease Institute, Nagasaki University Graduate School of Biomedical Sciences, [6]Division of Biomedical Engineering for Health and Welfare, Tohoku University Graduate School of Biomedical Engineering, [7]Department of Histology and Cell Biology, Faculty of Medicine, Kagawa University, [8]Institute of Basic Medical Research, International University of Health and Welfare

Background: Mutations in the *dysferlin* gene are responsible for adult-onset, progressive, and recessively inherited muscular dystrophies called dysferlinopathy, which has two main phenotypes: Miyoshi myopathy and limb-girdle muscular dystrophy type 2B. The dysferlin protein is expressed in the plasma membrane of skeletal muscles and has been reported to be involved in calcium-mediated membrane fusion and repair. We previously reported over 50 different mutations across the entire *dysferlin* and are focusing on these dysferlin and dysferlin-related proteins. **Objective**: To identify the binding partners of dysferlin, which can be working as the similar functional role, would be possible therapeutic targets to compensate the functional loss of the mutated dysferlin. **Methods and Results**: Using recombinant proteins and affinity purification methods combined with LC/MS/MS, we found a protein X that binds to a specific region of dysferlin. Next, using an *ex vivo* laser-injury experiment, we revealed that protein X is essential for the membrane repair of sarcolemmal damage in skeletal muscle fiber and the injury-triggered accumulation of the protein X was regulated by dysferlin. Moreover, an X-activating compound rescued the impairment of the damaged membrane repair in immortalized myoblasts established from the dysferlin-deficient patients and improves muscular dystrophy in a zebrafish model of dysferlinopathy. **Conclusions**: The protein X is required for plasma-membrane repair and a potential therapeutic target for dysferlinopathy.

AP-01-6 Creating mice models for sporadic Parkinson's disease based on its genetic risk factors

○Masashi Ikuno[1], Hodaka Yamakado[1], Hisako Akiyama[2], Yoshio Hirabayashi[2], Ryosuke Takahashi[1]
[1]Kyoto University, [2]Institute of Physical and Chemical Research

[Background] An animal model is essential not only to explore the pathogenesis of Parkinson's disease (PD) but to search the biomarker and validate the candidate drugs of PD. [Objective] In this study, we try to generate a new appropriate animal model of PD. [Material and Methods] Alpha synuclein（α-syn）is important for the pathogenesis of PD. We previously generated α-syn bacterial artificial chromosome (BAC) transgenic mice harboring the entire human α-syn gene and its gene expression regulatory regions. They expressed 2.7-fold amount of α-syn with similar expression pattern to endogeneous α-syn. They manifested decreased anxiety-like behaviors which may reflect non-motor symptoms, but did not show dopaminergic neuronal loss. Recent genetic study showed that PD patients have higher prevalence of heterozygous GBA mutation, and reduced activity of GBA is presumed to affect accumulation of α-syn, although underlysing precise mechanisms remain unclear. Here, we crossed wild-type α-syn BAC tg mice with GBA heterozygous knockout mice to make PD mice model relevant to human PD. [Results] These mice showed more pathogenic α-syn species such as phosphorylated α-syn and showed dopaminergic neuronal cell loss. Interestingly, GBA activity was also decreased in α-syn BAC transgenic without GBA mutation. [Conclusion] These mice can be thought to be a mammalian neurodegeneration model of PD and show bidirectional pathogenic loop of GBA and α-syn in vivo model. We report characteristics of this model and results of some additional analysis including glycolipids associated with GBA.

AP-02-1 Development of a novel quantitative assay of p-tau and its application to the blood diagnosis of AD

○Harutsugu Tatebe[1,2], Takuma Ohmichi[1], Takashi Kasai[1], Masaki Kondo[1], Masaaki Waragai[3], David Allsop[4], Takahiko Tokuda[1,5]
[1] Department of Neurology, Kyoto Prefectural University of Medicine, [2] Department of Zaitaku (Homecare) Medicine, Kyoto Prefectural University of Medicine, [3] Department of Neurology, Higashi Matsudo Municipal Hospital, [4] Division of Biomedical and Life Sciences, Lancaster University, [5] Department of Molecular Pathobiology of Brain Diseases, Kyoto Prefectural University of Medicine

【背景】アルツハイマー病（AD）診断の髄液バイオマーカーの有用性が確立されており、特にリン酸化タウ蛋（p-tau）はAD患者脳に特異的に蓄積する病的蛋白質であり、認知症の発症と直接的に関連していることがわかっている。しかしながら、p-tauは、血液中には極微量しか存在していないために、これまではその定量はできなかった。【目的】超高感度デジタルアッセイSimoa（Single molecular array）法を用いて、血液中でp-tauを検出・定量する系を確立した。また、この新規の定量系がADの血液診断に有用か否かを検討した。【方法】抗tau抗体を用いた市販のt-tau定量Simoa系を改良して、新規のp-tau181の定量系を開発した。開発した定量系を用いて、AD20名と健常対象者15名の血漿中p-tauを測定した。さらに、ダウン症候群（DS）患者（n=20）の血漿中p-tauを測定し、年齢を一致させた健常人（n=22）と比較した。【結果】p-tau定量系の測定感度をフェムト（10^{-15}）グラム/mLのオーダーにまで向上させることに成功し、1測定に必要な血漿量は約50 μlであった。AD患者の血漿中p-tau濃度は、正常対象者と比較して有意に高値であった（AD群：0.171±0.166、対照群：0.0405±0.0756 pg/ml、p=0.0039）。また、この定量系の診断能力を判定するROC曲線のAUC値は0.786であり、少ないサンプル数にも関わらず中等度の正確性を有した。DS群では、正常対照群と比較して、血中p-tau値が有意に高値であり（p=0.0332）、DS患者の年齢と正の相関が認められた（R^2=0.4451, p=0.0013）。【結論】新規に開発したp-tau定量系で測定した血漿p-tau値は、認知症の責任病変となる大脳のアルツハイマー病理を反映するバイオーマーカーになると考えられる。

AP-02-2 Genetic and Phenotypic Profile of 112 Patients with X-linked Charcot-Marie-Tooth disease type 1

Jun-hui Yuan, ○Yusuke Sakiyama, Akihiro Hashiguchi, Masahiro Ando, Akiko Yoshimura, Yujiro Higuchi, Yuji Okamoto, Hiroshi Takashima
Department of Neurology and Geriatrics, Kagoshima University Graduate School of Medical and Dental Sciences

Objective To identify the frequency and mutational spectrum of patients with X-linked Charcot-Marie-Tooth disease type 1, and to demonstrate their phenotypic diversity of peripheral and central nervous systems. **Methods** From 2007 to September 2016, using three high throughput sequencing systems, we performed targeted gene panel sequencing on a Japanese nationwide cohort of 1,483 unrelated patients with clinically suspected Charcot-Marie-Tooth disease. We reviewed all patients with *GJB1* variants, and reevaluated their peripheral nervous system involvements with Charcot-Marie-Tooth Neuropathy Score for gender comparison. **Results** We identified 59 *GJB1* variants (including 24 novel variants) from 88 unrelated pedigrees, consisting of 34 female and 78 male patients. Between genders, age at onset differs significantly with a mean of 35.53±23.72 years in females and 21.56±17.63 years in males (p = 0.007). Male patients exhibit more significant disabilities of lower motor functions than females, other than upper extremities or sensory dysfunctions. Central nervous dysfunctions were found in 15 patients from 12 pedigrees. Therein, six patients from four pedigrees developed episodic symptoms, with dysarthria as the most common phenotype. **Conclusions** We demonstrate a relatively lower frequency (5.9%) of X-linked Charcot-Marie-Tooth disease type 1 in our cohort, and describe a detailed profile of gender difference on the basis of quantification of clinical and electrophysiological findings. We also present a broad spectrum of central nervous dysfunctions in 13.4% of our patients.

AP-02-3 Anti-glycolipid antibodies and clinical features in recurrent Guillain-Barré syndrome

○Naoki Kotsuki[1], Ayumi Uchibori[1], Hiroto Ito[2], Yuki Hatanaka[3], Atsuro Chiba[1]
[1] Department of Neurology, Kyorin University, [2] National Hospital Organization Nagoya Medical Center, [3] Department of Neurology, Teikyo University

【目的】Guillain-Barré症候群（GBS）再発例の臨床像及び抗体プロファイルを検討した。【方法】2010年1月-2017年4月期間に当科に抗体測定の依頼または自験例1076症例のうち再発が確認されたGBS 5例を対象とし、ガングリオシド単独抗原及び複合抗原に対する抗体をELISAで測定し、臨床像を解析した。【結果】性別は全例が男性、平均年齢は28.4歳、初回発症年齢は20～40歳。初回発症から再発までの平均期間は10.6年で1例は2回の再発を来した。初発時の臨床病型は4例がFisher症候群（FS）で、1例は眼球運動障害を伴うGBS（GBS-OP）であり、FS1例とGBS-OP1例は再発時も同じ臨床病型を呈し、FSのうち2例は再発時にBickerstaff型脳幹脳炎（BBE）を、2回再発FS例では2回目にFS、3回目にBBEを呈した。GT1aとcross reactionするIgG抗GQ1b抗体を4例で認め、そのうち3例でGQ1bを含む複合抗原に対する抗体が検出された。2回再発症例では全経過でGQ1b単独抗原に対する反応はみられず、GQ1b, GT1aを含む複合抗原との強い反応がみられ複合体抗体のバリエーションが再発毎に拡大した。また回復期・寛解期には抗体価は低下し再発時に再上昇していた。また深部感覚障害を伴う症例ではGD1b抗原関連の抗体が検出された。【結論】再発例は全例初発再発時ともに眼球運動障害を伴う抗GQ1b抗体が関連したFS関連病態であり、再発時には類似の抗体が検出され、同一スペクトラムの臨床病型を示す傾向がある。一方で再発時には重症化傾向があり、2回再発例ではBBE発症時に重症GBSに関連するとされるGD1b/GD1a、GD1b/GT1bに対する反応が新たにみられ、重症化への関与が示唆された。GD1b関連抗体は既報告同様深部感覚障害との関連が示された。再発時には類似病型を示しながらも、抗体プロファイルが臨床症状や重症度を規定している可能性がある。

AP-02-4 Heterogeneous histopathology in dermatomyositis with normal serum levels of creatine kinase

○Kenichiro Taira[1], Meiko Maeda[2], Naohiro Uchio[1], Kadoya Masato[3], Atsushi Unuma[1], Chiseko Ikenaga[1], Akatsuki Kubota[1], Shoji Tsuji[1], Tsuneyo Mimori[4], Jun Shimizu[1], Tatsushi Toda[1]
[1] Department of Neurology, Graduate School of Medicine, the University of Tokyo, [2] Department of Neurology, Federation of National Public Service Personnel Mutual Aid Associations Toranomon Hospital, [3] Division of Neurology, Department of Internal Medicine, National Defense Medical College, Saitama, [4] Department of Rheumatology and Clinical Immunology, Kyoto University, Kyoto, Japan.

[Background] The levels of creatine kinase (CK) can be normal in active dermatomyositis (DM). We aimed to study the histopathological features in DM with normal serum CK levels. [Methods] In the study of 286 adult patients muscle biopsied with DM rash, we included 42 patients pretreated with normal values of serum CK (nCK-DM). In addition to the light microscopic analysis, we observed the endothelial tubuloreticular profiles (TRPs) on the electron microscopy. As the control group, 100 consecutive DM patients with elevated CK were used. [Results]nCK-DM was less closely associated with cancer (14%, p = 0.009). They showed weakness (71%). Serum aldolase levels were high in 37%. Myositis-specific autoantibodies were present in 83%. Anti-MDA5-Ab was more prevalent (44%, p < 0.001), followed by anti-TIF1-γ-Ab (31%, p = 0.85). Cancer was not detected in TIF1-γ-nCK-DM (38%, p = 0.0012). Myopathological findings, which were compatible with DM showed major histocompatibility complex class 1 (MHC-I) overexpression (88%). The TRPs were observed in 88%. In comparison between MDA5-nCK-DM and TIF1-γ-nCK-DM, MDA5-nCK-DM showed slight myopathic changes (67%, p = 0.009). TIF1-γ-nCK-DM presented with perimysial inflammation (100%, p = 0.021), perifascicular atrophy (75%, p = 0.0017), and microvascular deposition of the complement membrane attack complex (50%, p = 0.012). [Conclusions] nCK-DM has the DM histopathology mainly characterized by the patterns of anti-MDA5 and -TIF1-γ-Abs. Our findings expand the knowledge of DM.

AP-02-5 Functional network features of visuoperceptual disturbances in Parkinson's disease

○Kazuya Kawabata[1,2], Hirohisa Watanabe[2], Reiko Ohdake[2], Kazuhiro Hara[1], Epifanio Bagarinao[2], Toshiyasu Kato[1], Aya Ogura[1], Michihito Masuda[1], Takamasa Yokoi[1], Takashi Tsuboi[1], Masahisa Katsuno[1], Gen Sobue[2,3]
[1] Department of Neurology, Nagoya University Graduate School of Medicine, [2] Brain and Mind Research Center, Nagoya University, [3] Research Division of Dementia and Neurodegenerative Disease, Nagoya University Graduate School of Medicine

[Objective] This study aims to clarify the functional connectivity changes in the brain of patients with visuoperceptual disturbances in Parkinson's disease. [Methods] We evaluated 50 patients with non-demented Parkinson's disease (PD) and 32 healthy control subjects (HC) using 3T-MRI. Cognitive performances were assessed with the Mini-Mental State Examination, the Addenbrooke's Cognitive Examination - Revised and the Visual Object and Space Perception Battery (VOSP). We defined visuoperceptual disturbance as an incomplete letter score in VOSP below 95% confidence intervals from those of our normative data. For the investigation of resting-state networks, we conducted independent component analysis, dual-regression analysis and seed-based analysis using resting-state fMRI images. [Results] Among the 50 patients with PD, 18 patients (36%) had incomplete letter scores below the cut-off. These patients had significantly lower functional connectivity mainly in the cortices around the calcarine sulcus located within the primary visual network ($p < 0.05$, corrected for multiple comparisons), which consists of primary and secondary visual cortices. Seed-based analysis showed that functional connectivity between these aberrant regions and the surrounding amygdala regions negatively correlated with incomplete letter scores ($r = -0.53$, $p = 6.6\times10^{-3}$). [Conclusions] Our study suggested that lower functional connectivity within the primary visual network, and higher connectivity between the visual cortex and the amygdala, were closely associated with visuoperceptual disturbances in PD.

AP-02-6 Mutational analysis of AARS2 in adult-onset leukoencephalopathy lacking CSF1R mutation

○Naomi Mezaki[1,2], Norikazu Hara[1], Tamao Tsukie[1], Toshiyasu Ogata[3], Toru Baba[4], Takeshi Miura[1,2], Takanobu Ishiguro[1,2], Hiroaki Nozaki[5], Kensaku Kasuga[1], Yoshio Tsuboi[3], Etsuro Mori[4,6], Osamu Onodera[2], Takeshi Ikeuchi[1]
[1] Department of Molecular Genetics, Brain Research Institute, Niigata University, [2] Department of Neurology, Brain Research Institute, Niigata University, [3] Department of Neurology, Faculty of Medicine, Fukuoka University, [4] Department of Behavioral Neurology and Cognitive Neuroscience, Tohoku University Graduate School of Medicine, [5] Medical Technology, Graduate School of Health Sciences, Niigata University, [6] United Graduate School of Child Development, Osaka University

[Objective] Recently, mutations in *alanyl-transfer RNA synthetase 2* (*AARS2*) were identified in patients with adult-onset leukoencephalopathy (AARS2-leukoencephalopathy: AARS2-L) sharing similar clinical and neuroimaging features with adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP) due to *colony stimulating factor 1* (*CSF1R*) mutations. We aimed to identify *AARS2* mutations in patients with adult-onset leukoencephalopathy lacking *CSF1R* mutations and characterize the clinical and neuroimaging features. [Methods] Genetic analysis of *AARS2* was performed in 64 patients with adult-onset leukoencephalopathy who did not carry *CSF1R* mutations. Clinical and MR imaging features were retrospectively analyzed in patients with AARS2-L. [Results] We found novel *AARS2* mutations in 2 female patients. Patient 1 was homozygous for missense mutation in aminoacylation domain, and patient 2 was compound heterozygous for missense and frameshift mutations in aminoacylation domain of *AARS2*. The age at onset was 37 and 51 years. An initial symptom was cognitive decline in both patients. Patient 1 showed ataxia and pyramidal signs. Patient 2 exhibited parkinsonism and pyramidal signs. MR imaging exhibited bilateral white matter changes with frontoparietal predominance. Diffusion restricted lesions were detected in subcortical white matter on diffusion weighted image. [Conclusion] We report 2 patients with AARS2-L carrying novel *AARS2* mutations. AARS2-L should be considered in patients with adult-onset leukoencephalopathy lacking *CSF1R* mutation.

APe-01-1 Association analysis of SNPs near the DYT3 locus to dystonic symptoms in XDP

○Gerard Raimon M. Saranza[1], Derick P. Sumalapao[2], Aloysius A. Domingo[3], Paul D. Pasco[1], Roland Dominic G. Jamora[1], Lilian Lee[4], Anna Westenberger[3], Christine Klein[3]
[1] Department of Neurosciences, Philippine General Hospital, Philippines, [2] Department of Biology, College of Science, De La Salle University, Philippines, [3] Institute of Neurogenetics, University of Lubeck, Lubeck, Germany, [4] Child Neuroscience Center, Philippine Childrens Medical Center, Quezon City, Philippines

BACKGROUND: X-linked Dystonia-Parkinsonism (XDP, DYT3, Lubag Disease, OMIM # 314250) exhibits variability with respect to age at onset and with symptoms that range from very mild signs to severe forms of the disease. The considerable phenotypic variability seen in XDP suggests the presence of disease modifiers, such as genetic factors influencing disease expression. OBJECTIVE: We aimed to elucidate whether single nucleotide polymorphisms within the XDP locus affect the phenotypic expression of XDP. METHODS: 280 genetically confirmed XDP patients were included in the study. Logistic regression analysis was done to evaluate the association between phenotypes and four SNPs of interest: ChrX:71102421C>G, rs41484056, rs41438158 and ChrX:71653235C>T. Student t tests were used to compare continuous quantitative outcome measures while Z-test was used to compare proportions of patients. RESULTS: The ChrX:71102421C>G polymorphism had a significant effect on the presence of neck/shoulder dystonia (p=0.000). Logistic regression analysis showed that none of the SNPs influence age at onset of illness. Likewise, there was also no association seen between any of the SNPs and the initial symptom (whether dystonia or parkinsonism) and the region of initial dystonic manifestation. CONCLUSION: Significant association was seen between ChrX:71102421C>G and the presence of neck/shoulder dystonia. This finding may indicate that genetic factors influence disease expression in XDP and hence, the phenotypic variability.

APe-01-2 Altered gamma delta T cell repertoire correlates with disability in untreated multiple sclerosis

○Maimaitijiang Guzailiayi[1], Koji Shinoda[1], Mitsuru Watanabe[1], Yuri Nakamura[1], Katsuhisa Masaki[1], Takuya Matsushita[1], Ryo Yamasaki[1], Yasunobu Yoshikai[2], Jun-ichi Kira[1]
[1] Department of Neurology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan, [2] Division of Host Defense, Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan

Purpose: We reported that the deletion-type copy number variation at T cell receptor (TCR) α and γ loci increased the susceptibility to multiple sclerosis (MS), which suggested a deviated rearrangement of TCR γ and δ. We aimed to clarify the alteration of $\gamma\delta$ T cell repertoire in MS patients. **Methods:** Comprehensive flow cytometric immunophenotyping was performed in 30 MS patients with no disease-modifying therapies (DMTs), 31 MS patients treated with interferon (IFN)-β and 23 healthy controls (HCs). **Results:** The frequencies of regulatory $CD4^+$ T (Treg) cells ($CD25^+CD127^{low/-}$) among $CD4^+$ T cells, $V\delta2^+V\gamma9^+$ cells in $\gamma\delta$ T cells ($CD3^+TCR\gamma\delta^+TCR\alpha\beta^-$) were significantly decreased in MS patients with no DMTs compared to HCs (p=0.010, p=0.002, respectively). In MS patients under IFN-β, the frequencies of Treg cells, $V\delta2^+V\gamma9^+$ $\gamma\delta$ T cells were decreased (p=0.041 and p<0.001, respectively), but $V\delta1^-V\delta2^-V\gamma9^-$ $\gamma\delta$ T cells were increased compared to HCs (p<0.0001). The $V\delta1/V\delta2$ ratio was significantly elevated in both MS patients with no DMTs and those treated with IFN-β compared with HCs (p=0.003, p=0.001, respectively). The frequency of $V\delta2^+V\gamma9^+$ cells in $\gamma\delta$ T cells negatively correlated with disease severity defined by Expanded Disability Status Scale scores in MS patients with no DMTs (r=-0.504, p=0.005), but not in those treated with IFN-β (r=0.002, p=0.992). **Conclusion:** MS patients had distinct $\gamma\delta$ T cell repertoire compared to HCs and the altered $\gamma\delta$ T cell parameters were robustly associated with disease severity only in MS patients with no DMTs.

APe-01-3 GWAS based on ATN system identifies new susceptibility loci for Alzheimer disease

○Chen-chen Tan, Jin-Tai Yu, Lan Tan
Qingdao Municipal Hospital, School of Medicine, Qingdao University, China

Purpose: To identify new susceptibility loci for Alzheimer's disease (AD) and provide clues of the mechanisms through which these novel variants might be acting. **Method** We conducted a case-control genome-wide association studies (GWAS) based on ""A/T/N"" system from 699 participants in Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort. Meanwhile, we studied the novel loci with MRI measures, abnormal glucose metabolism, and β-amyloid (Aβ) deposition on neuroimaging in ADNI database. **Results** Near genome-wide significant effect was observed for rs10210302 within the ATG16L1, rs882908 within the CPPED1, rs4513478 within the NLGN1, rs17836440 within the WDFY4 and rs80201685 within the FRY. Meanwhile, we observed relationships between all these novel loci and Aβ retention. SNPs at rs10210302 within the ATG16L1 and rs17836440 within the WDFY4 were both associated with the decline rate for cerebral metabolism rate of glucose. SNPs at rs10210302 within the ATG16L1 also showed marked association with volume of left presubiculum, fimbria and subiculum. The novel loci rs17836440 within the WDFY4 was also associated with AD risk. Bioinformatics indicate that rs10210302 was transcription factor-binding site and significantly regulated ATG16L1 gene expression. **Conclusion** This study revealed 5 novel AD loci which have also been detected to be associated with one or a few established AD-related neuroimaging measures. Together the findings from this study can be used to inform future AD studies.

APe-01-4 MIR-132 EXPRESSION IN PLASMA OF PARKINSON'S DISEASE

○Zhaofei Yang, Tianbai Li, Min Wei, Song Li, Weidong Le
Center for Translational Research on Neurological Disease, 1st Afliated Hospital, Dalian Medical University, China

Purpose: microRNAs (miRNAs) are mall, evolutionary conserved and non-coding small RNAs involved in posttranscriptional gene regulation. miR-132 has been reported to regulate nuclear receptor related 1 protein (Nurr1), which plays a key role in the maintenance of the dopaminergic system of the brain and mutations in this gene have been associated with disorders like Parkinson's disease, schizophrenia, and manic depression. The miR-132 expression level in plasma of patients of PD, compared to neurological disease controls (NDC) and healthy controls (HC) has not been reported yet. Therefore, we determine whether miR-132 expression is altered in patients with PD we measured its expression in human plasma in 240 patients with PD, 218 HC, and 200 NDC by reverse transcription real-time quantitative PCR (RT-qPCR) in the form of single blind, using artificial synthetic external miRNA as control. miR-132 expression levels was significantly increased nearly 2-folds in patients with PD as compared with HC ($p < 0.01$) and has a increased trend as compared with NDC. When adjusted for gender, age, higher levels of miR-132 expression were associated with significantly increased risk for PD in men, and whose expressions were closely related with the disease stages and disease severity. The observed high miR-132 expression in plasma indicates possible systemic involvement in PD, and the finding may help identify individuals with PD and other disorders.miR-132 levels in plasma is expected to be used as a potential biomarker aiding in diagnosis and prognosis of PD.

APe-01-5 Rehabilitation and Readmission/Mortality Risks in Patients with Stroke or Transient Ischemic Attack

○Chu-ling Yen[1], Ku-Chou Chang[1,2,3], Hsuei-Chen Lee[4], Jen-Wen Hung[1,2]
[1] Kaohsiung Chang Gung Memorial Hospital, Taiwan, [2] Chang Gung University, Taiwan, [3] Yuh-Ing Junior College, Taiwan, [4] National Yang Ming University, Taiwan

Objective: The high-dose stroke rehabilitation induces greater functional improvement than the low-dose stroke rehabilitation. The impact of the dose of stroke rehabilitation on subsequent readmission/mortality risks remains unclear. The objective of this study was thus to investigate the associations between the dose of stroke rehabilitation and risks of 3 outcome events (OEs) in patients with stroke or transient ischemic attack (TIA). *Methods:* The Taiwan National Health Insurance database was used. Information on 4594 patients with first-ever acute stroke or TIA was collected with the averaged follow-up duration of 32 months. Three OEs were investigated: vascular readmissions/all-cause mortality (VE), all-cause readmissions/mortality (OE1), and all-cause mortality (OE2). Three doses of rehabilitation were used in Model 1 (None, Low-dose, and High-dose) and 4 doses were used in Model 2 (None, Inpatient plus/or Outpatient). *Results:* Comparing to None, Low-dose was related to lower risks of VE (Hazard Ratio (HR) 0.77; 95% Confidence Interval (CI) 0.68-0.87) and OE1 (HR 0.77; 95% CI 0.71-0.84), but not OE2 (HR 0.91; 95% CI 0.77-1.07). High-dose was related to lower risks of VE (HR 0.68; 95% CI 0.58-0.79), OE1 (HR 0.79; 95% CI 0.71- 0.88), and OE2 (HR 0.56; 95% CI 0.44-0.71). Furthermore, Inpatient plus Outpatient rehabilitation was related to the lowest risks of VE (HR 0.55; 95% CI 0.47-0.65), OE1 (HR 0.65; 95% CI 0.58-0.72), and OE2 (HR 0.45; 95% CI 0.35-0.59). *Conclusions:* Rehabilitation was associated with reduced readmission/mortality risks in patients with stroke or TIA.

APe-01-6 Interactions between caffeine intake and LRRK2 gene in Parkinson's Disease

○Yi Lin Ong[1,2], Xiao Deng[2], HuiHua Li[3], Prakash M Kumar[1,2], Pavanni Ratnagopal[1,2], Louis C.S. Tan[2], Wing Lok Au[2], Eng King Tan[1,2]
[1] DUKE-NUS Medical School, Singapore, [2] National Neuroscience Institute, Singapore, [3] Health Services Research, Singapore General Hospital, Singapore

Objective: We investigated the gene-environment interaction between caffeine consumption and genetic susceptibility to PD, specifically at the LRRK2 gene S1647T variant. Multiple susceptibility genes and environmental factors have been implicated in PD but gene-environment interactions have not been studied in detail. Caffeine has been associated with reduced PD risk. The leucine-rich repeat kinase 2 (LRRK2) gene has been linked to the autosomal dominant familial form of PD, and the S1647T variant is a risk factor for PD in the Chinese population. Methodology: A case control study of 2006 PD cases and 2688 healthy controls was conducted. Caffeine intake was assessed using a validated questionnaire. Genotyping of LRRK2 S1647T variant was carried out. Results: Compared to caffeine consumers with low genetic susceptibility, non-caffeine consumers with high genetic susceptibility (homozygous recessive mutants) had almost four times increased risk [OR 3.99, 95% CI (1.98, 8.04), p < 0.001], with greater effect seen in the Chinese population [OR 4.54, 95% CI (2.14, 9.62), p<0.001]. There was no significant dose-response interaction with quantitative caffeine intake. Conclusion: There is evidence that caffeine consumption significantly reduces the risk of PD in cases with high genetic susceptibility compared to those with low genetic susceptibility at the LRRK2 S1647T loci. Future studies can investigate the interactions with other genetic risk variants of PD.