## AO-01-1 Efficacy of bone marrow transplantation for adolescent/adult-onset cerebral/cerebello-brainstem ALD

○Takashi Matsukawa[1,2], Tomotaka Yamamoto[2], Akira Honda[3], Jun Mitsui[1], Hiroyuki Ishiura[2], Akihito Hao[2], Miho Matsukawa[2], Masaki Tanaka[4], Toji Miyagawa[2], Masashi Hamada[2], Yuji Takahashi[5], Kaori Sakuishi[2], Atsushi Iwata[2], Jun Shimizu[2], Jun Goto[6], Mineo Kurokawa[3], Shoji Tsuji[1], Tatsushi Toda[2]
[1] Department of Molecular Neurology, University of Tokyo, Graduate School of Medicine, Japan, [2] Department of Neurology, The University of Tokyo hospital, Japan, [3] Department of Hematology and Oncology, The University of Tokyo hospital, Japan, [4] Institute of molecular genetics, International university of health and welfare, Japan, [5] Department of Neurology, National Center of Neurology and Psychiatry, Japan, [6] Department of Neurology, International University of Health and Welfare Mita Hospital, Japan

**[Objective]** There are acummulating evidneces on the efficacy of hematopoietic stem cell transplantation (HSCT) for child-onset cerebral adrenoleukodystrophy (ALD) at early stages to stop disease progression. There are a few previous reports of HSCT for adult-onset cerebral ALD. To evaluate the efficacy of HSCT for adult cerebral ALD, we compared clinical outcomes of adolescent/adult-onset ALD patients who underwent bone marrow transplantation (BMT) with those of patients who did not receive HSCT. **[Methods]** We conducted BMT for 12 adolescent/adult-onset cerebral or cerebello-brainstem ALD patients at early stages with nonmyeloablative regimens (group 1). In 8 ALD patients, HSCT was not conducted because of advanced stages or patients' will (group 2). We conducted survival analysis using Kaplan-Meier plot from onset of cerebral/cerebellar/brainstem lesions and log-rank test. This study was approved by IRB. **[Results]** The median ages at onset were 24.5 (range: 16-42) and 34.5 (18-76) in groups 1 and 2, respectively. In group 1, symptoms originated from brain and white matter lesoins on brain MRI stopped or partially improved after BMT with no fatality. In group 2, symptoms progressed and 5/8 patients died of disease progression in a median period of 66.0 (16.0-104.1) months after onset of cerebral/cerebellar/brainstem lesions. Survival probability was significantly higher in group 1 than in group 2 (P=0.0189). **[Conclusions]** BMT for adolescent/adult-onset ALD was effective to stop disease progression. Clinical course after BMT was better than patients who did not undergo BMT.

## AO-01-2 Safety and efficacy in 20 cases of POEMS syndrome

○Hiroshi Amino, Sonoko Misawa, Kazumoto Shibuya, Yukari Sekiguchi, Tomoki Suichi, Atsuko Tsuneyama, Youichi Suzuki, Keigo Nakamura, Satoshi Kuwabara
Chiba University Department of Neurology, Japan

【目的】POEMS症候群は形質細胞の異常に伴い末梢神経障害、浮腫、胸腹水、内分泌異常、M蛋白血症を伴う予後不良な疾患である。新規治療としてサリドマイドの有効性が確立されている。一方、サリドマイドへの抵抗例や急速に悪化する例もあり、サリドマイド以外の治療選択肢の開発も必要である。ボルテゾミブはプロテアソーム阻害を作用機序とした骨髄腫の新規治療薬である。本疾患における安全性と有効性について検討する。【方法】ボルテゾミブによる治療を行ったPOEMS症候群20例（男性10例、平均年齢53歳）を対象とした。ボルテゾミブ（皮下注射、1.0-1.3mg/㎡、1-2回/週）・デキサメタゾン（20mg/day、day1,2,4,5,8,9,11,12）療法を施行した。臨床症状、血清VEGF値、有害事象について検討した。【結果】ボルテゾミブを選択した理由は、治療抵抗（n=4）、急性増悪（n=7）、重症（n=10）であった。11例は投与後3ヶ月以内に、血清VEGF値は正常域まで低下し、臨床症状も改善した。4例はボルテゾミブ療法への抵抗性を示し、代替治療の選択を必要とした。有害事象としては25％で末梢神経障害、10％でイレウス、血球減少、便秘を認めた。Grade 3以上の末梢神経障害を認めた例はいなかった。【MS1】治療中止を必要とした有害事象は、イレウス（n=1）例、間質性肺炎（n=1）であった。1例は原疾患の悪化によると考えられる臓器不全で死亡した。1例は治療終了から2年経過後も無治療で寛解を維持している。【結論】末梢神経障害、イレウスなどの有害事象の発出現には注意が必要だが、POEMS症候群においてボルテゾミブ療法は、速やかに寛解導入できる新たな治療選択肢になりうる。

## AO-01-3 Serum GFAP and neurofilament light as biomarkers of disease activity and disability in NMOSD and MS

○Mitsuru Watanabe[1], Yuri Nakamura[1], Zuzanna Michalak[2], Fumie Hayashi[1], Christian Barro[2], David Leppert[2], Noriko Isobe[3], Takuya Matsushita[1], Ryo Yamasaki[1], Jens Kuhle[2], Jun-ichi Kira[1]
[1] Department of Neurology, Graduate School of Medical Sciences, Kyushu University, Japan, [2] Neurology, Departments of Medicine, Biomedicine and Clinical Research, University Hospital Basel, [3] Department of Neurological Therapeutics, Graduate School of Medical Sciences, Kyushu University

[objective] Glial fibrillary acidic protein (GFAP) and neurofilament light chain (NfL) are intermediate filaments of astrocytes and neurons, respectively. The aim of this study is to investigate whether serum GFAP (sGFAP) and NfL (sNfL) levels are related to disease activity and disability in neuromyelitis optica spectrum disorders (NMOSD) and Japanese multiple sclerosis (MS) patients. [Methods] GFAP and NfL levels in CSF and sera were measured in healthy controls (HC, n=49; 49 sera), NMOSD (n=33; 42 CSF and 102 sera) and MS patients (n=49; 53 CSF and 91 sera) by ultrasensitive single-molecule array (Simoa) assay. The associations of sGFAP and sNfL levels with clinical parameters was assessed. [Results] CSF and serum GFAP and NfL were strongly associated and both analytes in serum were higher in NMOSD than in HCs (p<0.0001, both). Moreover, sGFAP in NMOSD were higher than those in MS patients (median: 207.7 versus 121.1 pg/ml, p<0.001). In NMOSD, sGFAP were increased by recent relapse (540.9 versus 152.9 pg/ml, p<0.001). In multivariate analyses, sGFAP and sNfL were associated with Expanded Disability Status Scale (EDSS) scores in NMOSD (p=0.026 and 0.001, respectively). sNfL levels in MS were associated with EDSS score and presence of recent relapse (p=0.019 and 0.009, respectively). A higher sGFAP/sNfL ratio at relapse could divide NMOSD from MS with a sensitivity of 73.0% and specificity of 75.8%. [Conclusions] sGFAP and sNfL are biomarkers of disease activity and disability in NMOSD and MS, and sGFAP/sNfL ratio at relapse is a potential diagnostic marker for NMOSD.

## AO-01-4 Relationship between cerebral small vessel disease and serum titer to periodontal pathogens

○Yuji Shiga[1], Naohisa Hosomi[1], Tomohisa Nezu[1], Hiromi Nishi[2], Shiro Aoki[1], Masahiro Nakamori[3], Kenichi Ishikawa[1,3], Hiroki Ueno[1], Hirofumi Maruyama[1]
[1] Department of Clinical Neuroscience and Therapeutics, Hiroshima University Graduate School of Biomedical and Health Sciences, Hiroshima, Japan, [2] Department of General Dentistry, Hiroshima University Hospital, Hiroshima, Japan, [3] Department of Neurology, Suiseikai Kajikawa Hospital, Hiroshima, Japan

【目的】歯周病を含めた口腔内環境は脳血管疾患や心疾患のリスク因子と考えられており，我々は，急性期脳梗塞患者を対象に歯周病菌血清抗体価と内頚動脈の内膜中膜複合体の肥厚，心房細動との関連について報告してきた．一方，脳微小出血や白質病変といった脳小血管病も脳卒中のリスク因子である．今回，急性期脳卒中患者における歯周病菌血清抗体価を用いて脳小血管病との関連の有無について検討をおこなった．【方法】当院，および共同研究機関に急性期脳卒中で入院となった患者を対象とした．歯周病菌の血清抗体価は脳卒中発症3日以内に採取された検体を用い，9菌種16菌体を測定した．抗体価は対数変換をおこない，すべての対象患者の平均から1SD以上を陽性と定義した．MRIのT2*画像を用いて脳微小出血の数を評価した．白質病変の重症度はFazekas分類を用いてFLAIR画像で評価し，Periventricular Hyperintensity (PVH) とDeep and Subcortical White Matter Hyperintensity (DSWMH) に関して0-1を軽症，2-3を重症と定義した．【結果】627例（脳梗塞522例，脳出血105例）を対象とした．脳微小出血を有する患者（n=315）は有さない患者（n=312）と比較して*C. rectus*の抗体陽性率が高かった（14.6% vs. 8.65%, P=0.0246）．その他の歯周病菌の陽性率には差を認めなかった．また，年齢，性，心血管リスク因子を含めた患者背景因子で補正後もC.rectus抗体陽性は脳微小出血の有無に関連した（odds ratio [OR] 2.04, 95% CI 1.19-3.48, P=0.009）．今回の検討では深部白質病変に関しては血清抗体価との関連はなかった．【結論】歯周病抗体の中で、*C. rectus*抗体陽性であることは脳微小出血の存在と関連した．

## AO-01-5 Voxel-based QSM analysis as an imaging biomarker for mild cognitive impairment in Parkinson disease

○Yuto Uchida[1,2], Hirohito Kan[3], Keita Sakurai[4], Shoji Kawashima[1], Yoshino Ueki[5], Kohsuke Kudo[6], Noriyuki Matsukawa[1]
[1] Department of Neurology, Nagoya City University Graduate School of Medical Sciences, Japan, [2] Department of Neurology, Toyokawa City Hospital, Japan, [3] Department of Radiology, Nagoya City University, Japan, [4] Department of Radiology, Teikyo University School of Medicine, Japan, [5] Department of Rehabilitation Medicine, Nagoya City University, Japan, [6] Department of Diagnostic and Interventional Radiology, Hokkaido University, Japan

[Objective] Brain iron accumulation has been proposed as one of the pathomechanisms in Parkinson disease (PD). This study aimed to examine the whole-brain pattern of iron accumulation associated with cognitive impairment in patients with PD, using voxel-based quantitative susceptibility mapping (QSM) analysis. [Methods] We enrolled 24 patients with PD and mild cognitive impairment (PD-MCI), 22 patients with PD and normal cognition (PD-CN), and 20 age-matched healthy controls (HC) in this case-control study. All participants underwent global cognitive and physical assessments and brain MRI. Using a combined method of voxel-based morphometry and QSM, we compared the voxel-wise magnetic susceptibility of the whole brain between the groups and analyzed its correlation with the cognitive and behavioral data. [Results] The PD-MCI group had lower Montreal Cognitive Assessment (MoCA) scores than the PD-CN and HC groups. There were no gray matter volumetric differences between the groups. In contrast, the voxel-based QSM analysis showed that the PD-MCI group had significantly higher QSM values in the precuneus, caudate head, inferior temporal gyrus, and anterior cingulate gyrus than did the PD-CN group. These QSM values were negatively correlated with MoCA scores in all participants (precuneus; r = -0.466; p < 0.001, caudate head; r = -0.453; p < 0.001). [Conclusions] This study suggests that cognitive impairment in PD is associated with cerebral iron burden and highlights the potential of QSM as an auxiliary biomarker for early evaluation of cognitive decline in patients with PD.

## AO-01-6 Multi-omics analysis reveals myasthenia gravis specific neuronal molecular regulation patterns

○Yoshiaki Yasumizu, Tatsusada Okuno, Makoto Kinoshita, Kotaro Ogawa, Naganari Ohkura, Hideki Mochizuki
Osaka University, Japan

重症筋無力症においては、約３割の患者で胸腺腫の合併が知られている。また、これら胸腺腫 においては抗AchR抗体に加えて、様々な神経関連分子に対する自己抗体の存在も知られている。しか し、胸腺機能と重症筋無力症との関わりを捉えるのはこれまで困難であった。そこで今回、我々はThe Cancer Genome Atlas (TCGA) に登録されている126例の胸腺腫患者のデータを用い、多層オミックスデー タ解析による重症筋無力症の病態メカニズム解明を試みた。【方法】解析にはTCGAに登録されてい る126例の胸腺腫サンプルのうち欠損値のない113例のゲノム変異、mRNA発現、DNAメチル化プロファ イルを用いた。Limma及びTCGA biolinksを用いた重症筋無力症の既往を有する群 (n=34) と有しない 群 (n=79) の比較解析及び、WGCNAを用いたネットワーク解析を行った。【結果】 重症筋無力症合 併胸腺腫群では非合併群に比し、有意にneurofilament、GABAA受容体、Ryanodine受容体や、新規の神経関 連分子が高発現しており、これら遺伝子領域のDNA脱メチル化亢進も認められた。逆に体細胞変異や浸 潤免疫細胞のプロファイルには重症筋無力症発症と相関が無いことがわかった。更に全サンプルを元に作られた遺伝子共発現ネットワークを構築すると、４つの主要なモジュールが認められ、それぞれB細 胞の活性化、T細胞の活性化、器官形成、神経発達に関連する遺伝子が集積していた。中でも神経発達関 連モジュールは高度に重症筋無力症発症と相関していた。【結論】重症筋無力症合併胸腺腫において は、神経発達関連分子の制御が病態に深く関わっている可能性が高いことを明らかにした。

## AO-02-1 Alpha-synuclein propagation via olfactory pathway in non-human primate model

○Masanori Sawamura[1], Hirotaka Onoe[2], Hideo Tsukada[4], Kaoru Isa[3], Norihito Uemura[1], Tadashi Isa[2,3], Ryosuke Takahashi[1]
[1] Department of Neurology, Graduate school of Medicine, Kyoto University, Japan, [2] Human Brain Research Center, Kyoto University Graduate School of Medicine, [3] Department of Physiology and Neurobiology, Graduate School of Medicine, Kyoto University, [4] Central Research Laboratory, Hamamatsu Photonics KK

Objective: Parkinson's disease (PD) is a neurodegenerative disease characterized by α-synuclein (α-Syn) aggregates, called Lewy bodies. The α-Syn aggregates are believed to propagate in the brain like prion via two major pathways: the olfactory and vagal pathways. In this study, we analyzed pathological progression by α-Syn fibrils injected into the olfactory bulb (OB) of common marmosets and measured regional brain activity by [18F]FDG-PET (2-deoxy-2-[18F]fluoro-D-glucose - positron emission tomography). Methods: α-Syn fibrils solution was stereotaxically injected into unilateral OB of two female and two male marmosets (about two years old) under anesthetized condition. Three or six months after the injection, we measured regional brain activity by [18F] FDG-PET and then sacrificed. The brains were immunostained with anti-phosphorylated α-Syn (p-α-Syn), anti-ubiquitin (Ub) and anti-p62 antibodies. Results: Wide and progressive spreading of p-α-Syn positive aggregates, which were also positive for Ub and p62, were observed in the ipsilateral OB, amygdala and entorhinal cortex suggesting the propagation of α-Syn pathology along with anatomically connected neurons over 6 months. Importantly, the OB, amygdala and entorhinal cortex are related to PD prodromal symptoms: hyposmia, anxiety and cognitive dysfunction, respectively. In addition, [18F]FDG-PET study revealed the hemisphere hypoactivity. Conclusions: We created the marmoset model showing propagation of α-Syn fibrils via olfactory pathway. This could potentially be a non-human primate model of prodromal PD.

## AO-02-2 BBB-crossing drug delivery system by modulating tight junction at brain microvascular endothelium

○Satoshi Zeniya[1], Hiroya Kuwahara[1], Kaiichi Daizo[1], Akihiro Watari[2], Masuo Kondoh[2], Kie Yoshida-tanaka[1], Hidetoshi Kaburagi[3], Ken Asada[1], Tetsuya Nagata[1], Masahiro Nagahama[4], Kiyohito Yagi[2], Takanori Yokota[1]
[1] Department of Neurology and Neurological Science, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University (TMDU), Japan, [2] Graduate School of Pharmaceutical Sciences, Osaka University, [3] Department of Orthopaedic and Spinal Surgery, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University (TMDU), [4] Department of Microbiology, Faculty of Pharmaceutical Sciences, Tokushima Bunri University

[Objective] Antisense oligonucleotide (ASO)-based therapeutics are now a powerful means of treating intractable neurological diseases. However, safe yet efficient methods of delivering ASOs across the blood-brain barrier (BBB) to the central nervous system remains to be unsolved. [Methods] We prepared angubindin-1, a binder to the tricellular tight junction, to modulate paracellular transport between brain microvascular endothelial cells in the BBB. Then we examined the effects of angubindin-1 on the delivery of ASO across the BBB in mice by histological and biochemical analyses. [Results] Intravenously injected angubindin-1 increased the permeability of the BBB and enabled transient delivery of subsequently administered ASO into the brain and spinal cord, leading to silencing of a target RNA without any overt adverse effects. The delivery of subsequently administered ASOs to the brain was verified, and silencing of the target RNA in the central nervous system was shown to be dependent on the dosage of angubindin-1 and ASOs, as well as the interval of administration between them. Bicellular tight junction binders did not produce such a silencing effect, suggesting that the tricellular tight junction is likely a better target for the delivery of ASOs than the bicellular tight junction. [Conclusions] This proof-of-concept study demonstrated that our delivery strategy of modulating the tricellular tight junction in the BBB via angubindin-1 provides a novel avenue of research for the development of ASO-based therapeutics for the treatment of neurological disorders.

## AO-02-3 Targeting Tyro3 ameliorates a model of PGRN-mutant FTLD-TDP via tau-mediated synaptic pathology

○Kyota Fujita, Xigui Chen, Hidenori Homma, Kazuhiko Tagawa, Hitoshi Okazawa
Department of Neuropathology, Medical Research Institute, Tokyo Medical and Dental University, Japan

[Objective] Mutation in the progranulin (PGRN) gene cause tau pathology-negative and TDP43 pathology-positive phenotype of frontotemporal lober degeneration (FTLD-TDP). To date, it remains unclear whether aggregation of TDP43 is indispensable for the initiation of pathology. Furthermore, the molecules which can initiate the pathology prior to TDP43 aggregation is not known, and it remains unclear how functional changes in synapses occur in FTLD. [Method] To investigate the molecular mechanism of PGRN-linked FTLD, we newly generated a knock-in mouse carrying the R504X mutation (PGRN-KI). Also, we performed phosphor-proteome analysis to verify the key phosphoprotein which is responsible the pathology of FTLD. [Result] Our novel PGRN-KI mice exhibit phenotypes resembling human FTLD. Using this new model, we identified the novel phosphorylation site of tau that is linked to the initiation of synapse pathology prior to TDP43 aggregation. Furthermore, we newly discovered that PGRN level in mutant mice activated Tyro3 signaling, leading to PKC and MAPK activation, mislocalization of Ser203-phosphorylated tau, and reduction in the number of synaptic spines. Administration of a PKC inhibitor, b-raf inhibitor, or knockdown of molecules in the Gas6-Tyro3-tau axis rescues spine loss and cognitive impairment of PGRN-KI mice. [Conclusion] These results suggest that targeting of early-stage and aggregation-independent tau signaling represents a promising therapeutic strategy for FTLD.

## AO-02-4 A novel cell transplantation therapy for ALS using OPCs expressing scFv recognizing misfolded SOD1

○Sumio Minamiyama[1,2], Madoka Sakai[3,4], Yuko Yamaguchi[2], Ryota Hikiami[1,2], Yoshitaka Tamaki[1], Akemi Shodai[1], Akiko Makino[3,4], Takakuni Maki[2], Keizo Tomonaga[3,4], Ryosuke Takahashi[2], Makoto Urushitani[1]
[1] Department of Neurology, Shiga University of Medical Science, Japan, [2] Department of Neurology, Kyoto University Graduate school of Medicine, Japan, [3] Laboratory of RNA Viruses, Department of Virus Research, Institute for Frontier Life and Medical Sciences (InFRONT), Kyoto University, Japan, [4] Department of Mammalian Regulatory Network, Graduate School of Biostudies, Kyoto University, Japan

[Objective] Mutations in superoxide dismutase1 (SOD1) are a leading cause of familial ALS. A number of studies have demonstrated that the misfolded forms of SOD1 could spread from cell to cell via diverse mechanisms. 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. These evidences led us to test a combination therapy of OPC transplantation and antibody targeting extracellular SOD1 to improve aberrant oligodendrocyte environment and to prevent non-cell-autonomous cells death. [Method] cDNA for tandem single chain of a monoclonal antibody (clone X) recognizing misfolded SOD1 specifically (scFv-X) was constructed, and was subcloned into a Borna disease virus (BoDV) vector, an advantage of which is episomal multiplication in the recipient cells. Primary OPCs was infected by the BoDV carrying scFv-X (scFv-X-OPC). We injected intrathecally to SOD1H46R rats OPCs alone or scFv-X-OPCs and investigated their motor function and survival time. [Result]The mammalian expression cassette for scFv-X was constructed and was subcloned into BoDV vector. The scFv-X-OPC secreted scFv-X, which recognized misfolded SOD1 specifically. While single injection of OPC delayed onset and extended life span of rats, the efficacy of scFv-X-secreting OPCs were higher than OPC alone. [Conclusion] Our combination therapy of OPCs and scFv showed the effect to ameliorate SOD1-mediatied ALS. Further in vivo evaluation is required to validate the efficacy and the mechanisms of this therapy.

## AO-02-5 Wide distribution of alpha-synuclein oligomers in MSA brain detected by proximity ligation

○Hiroaki Sekiya[1,2], Hisatomo Kowa[1,3], Hinako Koga[1], Mariko Takata[1], Wataru Satake[1], Naonobu Futamura[4], Itaru Funakawa[4], Kenji Jinnai[4], Motonori Takahashi[5], Takeshi Kondo[5], Yasuhiro Ueno[5], Motoi Kanagawa[1], Kazuhiro Kobayashi[1], Tatsushi Toda[1,6]
[1] Division of Neurology/Molecular Brain Science, Kobe University Graduate School of Medicine, Japan, [2] Department of Neurology, Hyogo Prefectural Amagasaki General Medical Center, Japan, [3] Department of Rehabilitation Science, Kobe University Graduate School of Health Sciences, Japan, [4] Department of Neurology, National Hospital Organization Hyogo-Chuo Hospital, Japan, [5] Division of Legal Medicine, Department of Community Medicine and Social Health Science, Kobe University Graduate School of Medicine, Kobe, Japan, [6] Department of Neurology, Graduate School of Medicine, The University of Tokyo, Japan

[Objective] To reveal the distribution of alpha-synuclein (AS) oligomers as an early pathological change in multiple system atrophy (MSA) brains. [Background] Although severe neuronal loss is observed in MSA, neuronal inclusions (NIs) are rare compared to AS-positive glial cytoplasmic inclusions (GCIs), such that the pathological mechanism of neuronal loss in MSA is unclear. GCIs and NIs are late-stage pathological features relative to AS oligomers and may not represent early changes. To reveal the early pathology of MSA, it is necessary to examine the early aggregation of AS, i.e., AS oligomers. [Methods] We adopted a proximity ligation assay (PLA) to examine the distribution of AS oligomers in brain samples from patients with MSA and other diseases. We examined 5 MSA cases, 5 Parkinson's disease (PD) cases, and 9 control cases. [Results] MSA brains showed a wide distribution and abundant accumulation of oligomeric AS in neurons as well as oligodendrocytes of the neocortex. In the MSA group, 4 of 5 cases showed neuronal-clustered staining in 9 cortical regions, whereas neuronal-clustered staining was not observed in PD or control cases, except in 2 regions of 2 PD cases. Moreover, in several regions, the neuropil AS-PLA staining was significantly more abundant in MSA cases than in PD cases. [Conclusion] The wide distribution of AS oligomers in MSA brain neurons has not been described previously and indicates a pathological mechanism of neuronal loss in MSA.

## AO-02-6 CCR2-positive peripheral blood macrophages play protective roles in ALS mouse model

○Wataru Shiraishi, Ryo Yamasaki, Yuko Kobayakawa, Jun-ichi Kira
Department of Neurology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University, Japan

[Background] In human ALS as well as ALS model mice, microglia and astrocytes are activated and peripheral blood macrophages infiltrate in the spinal cord. Previous reports suggest an involvement of peripheral blood macrophages in ALS; however the role of these cells remain to be established. [Objective] To clarify the roles of C-C chemokine receptor type 2 (CCR2), a receptor for C-C motif chemokine 2 (CCL2), which chemoattracts peripheral blood macrophages, in the mechanism of ALS. [Methods] We generated $CCR2^{RFP/RFP}$/mutant superoxide dismutase-1 (G93A) transgenic (mSOD-1-tg) mice, in which $CCR2^{RFP}$ is expressed homozygously, resulting in a CCR2-deficient phenotype. We compared the disease course of CCR2-deficiet mSOD-1 mice with that of $CCR2^{RFP/WT}$/mSOD-1-tg mice (heterozygous phenotype) by behavioral and immunohistochemical analyses. [Results] CCR2-deficient mSOD-1-tg mice showed earlier onset and rapid progression (onset time: 10 vs. 11.5 weeks, $p<0.05$) compared with CCR2-positive mSOD-1-tg mice, resulting in a shorter survival time (164 vs. 172 days, $p<0.05$). Immunohistochemically, the sciatic nerves of CCR2-deficient mSOD-1-tg mice had significantly less macrophage invasion and more mutant SOD-1 protein deposition than CCR2-positive mSOD-1-tg mice, in which CCR2-positive foamy macrophages phagocytized mSOD-1 protein. [Conclusion] Deficiency of CCR2 results in more aggressive disease course and less macrophage infiltration into the peripheral nerves. Therefore, CCR2-positive macrophages act as mutant SOD-1 protein scavengers and play protective roles in the ALS mouse model.

## AP-01-1 NfL, tau, and TDP-43 in plasma and CSF as diagnostic and prognostic biomarkers of ALS

○Takuma Ohmichi[1], Takashi Kasai[1], Harutsugu Tatebe[2], Yuta Kojima[1], Yu-ichi Noto[1], Yukiko Tsuji[1], Makiko Shinomoto[1], Toshiki Mizuno[1], Takahiko Tokuda[1,3]
[1] Department of Neurology, Kyoto Prefectural University of Medicine, Japan, [2] Department for Medical Innovation and Translational Medical Science, Kyoto Prefectural University of Medicine, [3] Department of Molecular Pathobiology pf Brain Diseases, Kyoto Prefectural University of Medicine

【Objective】TDP-43, tau, and neurofilament light chain (NfL) levels in cerebrospinal fluid (CSF) are now regarded as candidates for diagnostic biomarkers for amyotrophic lateral sclerosis (ALS). The NfL levels in CSF and plasma are also expected to predict outcomes of patients with ALS. However, no study has comprehensively measured these markers and validated their diagnostic/prognostic values in both CSF and plasma. We aimed first to assess the ability of NfL, TDP-43, and tau in plasma and CSF to serve as diagnostic biomarkers in ALS and second to determine their prognostic values for clinical outcome. 【Methods】We enrolled a discovery cohort (30 patients with ALS and 29 age-matched disease controls) and a validation cohort (55 patients with ALS and 57 patients with ALS mimic syndromes). All measurements were done with the next generation immunoassay system (Simoa). Survival analysis was done with the Kaplan-Meier method, with death or initiation of permanent assisted ventilation as the endpoint. 【Results】Significant elevation of NfL levels in CSF, NfL levels in plasma, and TDP-43 levels in CSF in the ALS group compared to those of the controls was consistently observed across the discovery and validation cohorts. The other markers showed no significant difference between groups in the two cohorts. Higher NfL levels in CSF at recruitment were associated with shorter survival time. 【Conclusion】The level of NfL in CSF can serve as a diagnostic and prognostic biomarker in ALS. The level of TDP-43 in CSF and NfL in plasma may be useful diagnostic biomarkers of the disease.

## AP-01-2 Alteration of resting-state functional connectivity in patients with RBD from the J-PPMI cohort

○Noritaka Wakasugi[1], Hiroki Togo[1], Youhei Mukai[2], Noriko Nishikawa[2], Miho Murata[2], Yuji Takahashi[2], Takashi Hanakawa[1]
[1] Department of Advanced Neuroimaging, Integrative Brain Imaging Center, National Center of Neurology and Psychiatry, Japan, [2] Department of Neurology, National Center Hospital, National Center of Neurology and Psychiatry

【目的】αシヌクレイノパチーの前病段階における脳機能ネットワークの変化を明らかにするため，レム睡眠行動異常症(RBD)患者と高齢健常者に安静時脳機能結合MRI(rsfcMRI)を施行し，群間比較を行った．【背景】RBD患者では基底核ネットワークに異常が生じることが報告されているが，被験者数が少なく，基底核以外のネットワークに関する報告にも乏しい．【方法】終夜睡眠ポリグラフ(PSG)で診断された55名の特発性RBD患者(年齢 69.6±5.77 歳，男性 39 名，女性 16 名)を対象とした．RsfcMRIはエコープラナー法を用い，10分間の開眼安静下で撮像した．データ解析にはFSL (FMRIB's Software Library) を用いた．70名の年齢をマッチした高齢健常者集団から，group Indipendent Component Analysis (ICA) によって44の安静時ネットワークコンポーネントを抽出した後，dual regression解析によりRBD群と健常群のfunctional connectivity (FC) の群間比較，並びにRBD群のFCと臨床評価指標との相関解析を行った．閾値はp=0.05 (corrected across voxels) とした．【結果】RBD群では，基底核ネットワークの解析で中脳に (p=0.05)，感覚運動ネットワークの解析で右運動野に (p=0.02)，実行制御ネットワークの解析で右背外側運動前野に (p=0.04) 機能的結合の低下がみられた．基底核ネットワークの解析では，MDS-UPDRS part Ⅰのスコアと右前頭極との間に負の相関が見られた (p=0.04)．【結論】PSGで診断された，既報告より規模の大きいコホートで解析を行った．まず既報告同様，RBD患者の基底核ネットワークに異常を確認した．また新たな知見として，感覚運動ネットワーク，実行制御ネットワークにも異常が見られることを確認した．さらに，非運動症状が基底核ネットワークと右前頭極との機能的結合と相関することを確認した．今後も年1回のrsfcMRI撮影を継続し，今回認めた機能的結合の異常が時間経過とともにどう進行するかを縦断的に観察する．

## AP-01-3 Clinical subtypes and autoantibodies in chronic inflammatory demyelinating polyneuropathy

○Motoi Kuwahara[1], Taro Matsui[1,2], Keisuke Yoshikawa[1], Masaki Yamana[1], Yuta Fukumoto[1], Kenichi Kaida[2], Susumu Kusunoki[1]
[1] Department of Neurology, Kindai University Faculty of Medicine, Japan, [2] Department of Neurology, Anti-aging and Vascular Medicine, National Defense Medical College

【目的】慢性炎症性脱髄性多発根ニューロパチー (CIDP) はtypical CIDPとatypical CIDPに大別され電気生理学的特徴や治療反応性が異なる。今回我々はCIDPを病型別に解析して自己抗体との関連を検討した。【方法】2015年の1年間にCIDP病名で抗糖脂質抗体測定の依頼があった725例に対して後方視的に追加調査を行い、最終診断と病型、臨床データ、治療内容および治療反応性と抗糖脂質抗体について解析した。CIDPの診断はEFNS/PNSの診断基準に従いpossible以上を解析の対象とした。【結果】追加調査で153例においてCIDPの最終診断を確認した。内訳はtypical CIDP 103例 (67%)、DADS 14例 (9%)、MADSAM 14例 (9%)、pure motor 6例 (4%)、pure sensory 5例 (3%)、focal 4例 (3%)、分類不能または無回答が7例 (5%) であった。年齢の中央値はtypical CIDPが56歳であったのに対して、DADSは68歳、pure sensoryは74歳と比較的高齢であり、脳脊髄液蛋白の中央値はDADSが121mg/dlと最も高く、次にtypical CIDPが86.9mg/dl、pure motorが82.5 mg/dlであった。Typical CIDPではIVIgと副腎皮質ステロイドの有効率が86%と78%であったが、DADSでは69%と67%、pure motorでは60%と17%と低かった。治療後経過はtypical CIDPでは寛解単相型が32例 (32%)、再発寛解型が44例 (44%)、慢性進行型が24例 (24%) であったのに対して、pure motorは寛解単相型が0例 (0%)、再発寛解型が1例 (16%)、慢性進行性が5例 (83%) であった。抗糖脂質抗体は、IgMクラスはGM1抗体が27例、IgGクラスはLM1抗体が8例と最も多く検出された。IgM GM1抗体陽性例はtypical CIDP 17例、DADS 2例、MADSAM 2例、pure motor 4例、focal 3例であり、IgG LM1抗体陽性例はtypical CIDP 7例、DADS 1例であった。【結論】IgG LM1抗体陽性例の多くがtypical CIDPであり液性因子としてtypical CIDPの病態への関与が示唆された。一方、pure motorは多巣性運動ニューロパチーとの異同が問題と考えられた。

## AP-01-4 Next-generation tau PET imaging with 18F-PI-2620 in Alzheimer's disease (AD) and non-AD tauopathy

○Toshiki Tezuka[1], Keisuke Takahata[2,4], Hajime Tabuchi[2], Kyoko Mashima[1], Munenori Oyama[1], Kensuke Okada[1], Kei Funaki[2], Bun Yamagata[2], Morinobu Seki[1], Takahito Yoshizaki[1], Tadaki Nakahara[3], Yasuharu Yamamoto[2], Yasunori Sano[2], Yuki Momota[2], Mika Shiraiwa[2], Mamoru Shibata[1], Masahiro Jinzaki[3], Masaru Mimura[2], Jin Nakahara[1], Daisuke Ito[1]
[1] Department of Neurology, Keio University School of Medicine, Tokyo, Japan, [2] Department of Neuropsychiatry, Keio University School of Medicine, Tokyo, Japan, [3] Department of Diagnostic Radiology, Keio University School of Medicine, Tokyo, Japan, [4] Department of Functional Brain Imaging Research, National Institute of Radiological Sciences, National Institutes for Quantum and Radiological Science and Technology, Chiba, Japan

【Purpose】Tau aggregates are one of key pathologic features of AD and other neurodegenerative diseases. Recently, PET probes for tau have been developed for *in vivo* detection of tau accumulation, but have limitations in regard to off-target binding and lower ability to detect tau in non-AD tauopathies. The novel tau tracer, PI-2620 has a high binding-affinity and specificity for aggregated tau, and was indicated to have desirable properties for visualization of tau accumulation in both AD and non-AD tauopathies. The aim of the study was to assess the ability of PI-2620 to detect regional tau burden in the brain of patients. 【Methods】We recruited healthy volunteers, patients with AD, and non-AD tauopathies (PSP and CBS). PET with PI-2620 was performed, and its accumulation in the cortical and subcortical region was assessed by calculating standardized uptake value ratios, along with amyloid PET using $^{18}$F-florbetaben. 【Results】PI-2620 was accumulated in the retina, skin and substantia nigra in healthy volunteers, indicating off-target binding properties to melanin. In AD, focal retention of PI-2620 was evident in temporal and parietal lobes, precuneus, and cingulate. In PSP and CBS, elevated PI-2620 retention was evident in globus pallidus compared to controls and AD patients. 【Conclusions】AD patients clearly showed elevated PI-2620 signals in characteristic regions associated with neurofibrillary tangle deposition. The tracer retention in globus pallidus may possibly distinguish 4 repeat tauopathy from other diseases, although further cases are required to clarify this.

## AP-01-5 Evaluation of differential diagnosis in taopaties by 18F-THK5351 PET

○Michinori Ezura[1], Akio Kikuchi[1], Nobuyuki Okamura[2], Takafumi Hasegawa[1], Aiko Ishiki[3], Ryuichi Harada[4], Hiroyuki Arai[3], Kazuhiko Yanai[4], Shozo Furumoto[5], Manabu Tashiro[6], Yukitsuka Kudo[3], Atsushi Takeda[7], Masashi Aoki[1]
[1] Departments of Neurology, Tohoku University Graduate School of Medicine, Japan, [2] Division of Pharmacology, Faculty of Medicine, Tohoku Medical and Pharmaceutical University, [3] Departments of Geriatric and Respiratory Medicine, Institute of Development, Aging and Cancer, Tohoku University, [4] Departments of Pharmacology, Tohoku University Graduate School of Medicine, [5] Divisions of Radiopharmaceutical Chemistry, Cyclotron and Radioisotope Center, Tohoku University, [6] Divisions of Cyclotron Nuclear Medicine, Cyclotron and Radioisotope Center, Tohoku University, [7] Department of Neurology, Sendai Nishitaga Hospital

【目的】タウオパチーには、大脳皮質基底核症候群 (CBS)、進行性核上性麻痺 (PSP)、アルツハイマー病 (AD) といった疾患群が含まれ、タウオパチー内でも鑑別が難しいことがある。今回、タウPETトレーサーとして開発された$^{18}$F-THK5351 PETがタウオパチーの鑑別診断に有用であるか及び臨床スコアと$^{18}$F-THK5351集積の間に相関があるかどうかを検討した。【方法】CBS 9名、PSP 10名、AD 10名、健常者 (NC) 8名に対し、神経心理学検査、脳MRI、$^{18}$F-THK5351 PETを施行した。PMODを使用し、各個人の脳MRI画像を使用して関心領域を設定し、各領域と小脳皮質の比 (SUVR) を用いて$^{18}$F-THK5351集積を評価した。各疾患の鑑別にReceiver Operating Characteristic (ROC) 曲線を用いて、area under the curve (AUC) 値を算出した。また、神経心理学検査の臨床スコアと$^{18}$F-THK5351集積との相関も検討した。【結果】NC群と比較して、CBS群では中心前回、中心後回、淡蒼球、中脳で$^{18}$F-THK5351高集積を示し、一方PSP群では中脳、淡蒼球、AD群では下側頭回、紡錘状回で高集積を示した。いずれの疾患群もタウ関連病理に一致して、$^{18}$F-THK5351の高集積であった。CBS とPSPの鑑別では中心前回 (AUC = 0.900)、CBS とAD、PSPとADの鑑別には下側頭回 (AUC = 1.000) が有用であった。臨床スコアと$^{18}$F-THK5351集積には相関はなかった。【結論】CBS、PSP、AD患者群で$^{18}$F-THK5351の集積パターンは異なり、適切な関心領域を選択すると鑑別に有用であった。$^{18}$F-THK5351はタウオパチーの疾患鑑別に有用であることが示唆された。

## AP-02-1 Elucidation of early pathophysiology of spinal-bulbar muscular atrophy using disease specific iPSCs

○Kazunari Onodera[1,2], Daisuke Shimojo[1,3], Mayuko Yoda[1], Yasuharu Ishihara[3], Takuji Ito[1], Rina Okada[1], 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, Japan, [2] Department of Neurology, Nagoya University Graduate School of Medicine, Japan, [3] Department of Physiology, Keio University School of Medicine, Japan, [4] Research Division of Dementia and Neurodegenerative Disease, Nagoya University Graduate School of Medicine, Japan

**[Objective]** Spinal and bulbar muscular atrophy (SBMA) is an adult onset neuromuscular degenerative disease caused by the expansion of CAG repeat 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 fibroblasts of 4 SBMA patients and 3 age-matched controls. iPSCs were differentiated into motor neurons (MNs) for the pathophysiological analysis of SBMA. **[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 disease associated genes including *CALCA* (CGRP-1) and *TβR2*, suggesting that this model recapitulate early pathology of SBMA. We also identified ER stress inducers, tunicamycin and thapsigargin, as disease accelerating factors, which significantly enhanced the phenotypes of SBMA-MNs. Using this early disease model we performed microarray analysis, and identified several molecules which induced similar neurodegenerative phenotypes in control-MNs to those observed in SBMA-MNs, , **[Conclusions]** Using disease specific iPSCs, pre-aggregation early disease model of SBMA was established. With this model, uncovering early pathology of SBMA, and identification of novel biomarkers and therapeutic targets are expected.

## AP-02-2 Transplantation of human iPS cell-derived dopamine neural progenitor cells for Parkinson's disease

○Ryota Nakamura[1], Genko Oyama[1], Takayuki Jo[1], Kei-ichi Ishikawa[1,2], Risa Nonaka[1,2,3], Yasushi Shimo[1,4], Wado Akamatsu[2], Nobutaka Hattori[1]
[1] Department of Neurology, Juntendo University, Japan, [2] Center for Genomic and Regenerative Medicine, Juntendo University, [3] Department of Diagnosis, Prevention and Treatment Dementia, Graduate School of Medicine, Juntendo University, [4] Research and Therapeutics for Movement Disorders, Juntendo University

[Objective] To examine the efficacy and safety of midbrain dopaminergic progenitors derived from human iPS cells, as the cell source for Parkinson's disease model mouse. [Methods] To establish unilateral Parkinson model mice, we injected 6-OHDA into the striatum of immunodeficiency model mice. Severity of model mice were evaluated by rotation behavior of them after subcutaneous injection of apomorphine. We differentiated human iPS cells from healthy subjects into neurospheres containing dopamine neural precursor cells, using a neural differentiation protocol we recently established. After transplantating those cells, we continuously evaluated the apomorphine-induced rotation behavior of recipient. Four months after transplantation, we sacrificed the animals and analyzed their brain section to evaluate differentiation properties of transplanted cells in that. [Result] We established 36 6-OHDA injected PD model mice that met the criteria of PD symptoms. Then dopaminergic progenitors were transplanted into 18 of them and saline was injected into 18 of them as a sham group. No tumor formation was observed in the brain section at four months after transplantation. From the early stage after transplantation, transplantation group showed a decrease in rotation behavior and after 3 months it decreased significantly compared to sham group. [Conclusions] Highly-enriched dopaminergic neural progenitor cells differentiated from human iPS cells by our neural induction protocol could be transplanted safely even without purification by cell sorting and improved symptoms of PD model mice.

## AP-02-3 GBA haploinsufficiency accelerates alpha synuclein pathology in a prodromal PD model

○Masashi Ikuno[1], Hodaka Yamakado[1], Hisako Akiyama[2], Laxmi Kumar Parajuli[3], Katsutoshi Taguchi[4], Junko Hara[1], Norihito Uemura[1], Yusuke Hatanaka[1], Katsumi Higaki[5], Masaki Tanaka[4], Masato Koike[3], Ryosuke Takahashi[1]
[1] Department of Neurology Kyoto University Graduate School of Medicine, Japan, [2] Laboratory for Molecular Membrane Neuroscience, RIKEN Brain Science Institute, [3] Department of Cell Biology and Neuroscience, Juntendo University Graduate School of Medicine, [4] Department of Anatomy and Neurobiology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, [5] Division of Functional Genomics, Research Center for Bioscience and Technology, Faculty of Medicine, Tottori University

[Objective] Parkinson's disease (PD) is characterized by dopaminergic (DA) cell loss and the accumulation of pathological alpha synuclein (asyn), but its precise pathomechanism remains unclear. Recent studies have shown that a heterozygous mutation of *glucocerebrosidase* (*gba*) is one of the most important genetic risk factors in PD. In this study, we try to generate a new appropriate animal model of PD and investigate how *gba* haploinsufficiency contribute to PD. [Methods] We crossed asyn Bacterial Artificial Chromosome transgenic mice with *gba* heterozygous knockout mice. [Results] These double-mutant (*dm*) mice showed an increase in phosphorylated asyn in the regions vulnerable to PD, such as the olfactory bulb and dorsal motor nucleus of the vagus nerve. Only *dm* mice showed a significant reduction in DA cells in the substantia nigra pars compacta, suggesting these animals were suitable for a prodromal model of PD. *Dm* mice also showed an increased level of glucosylsphingosine without any noticeable accumulation of glucosylceramide, a direct substrate of GBA. In addition, the overexpression of asyn resulted in decreased GBA activity in mice, while *dm* mice tended to show an even further decreased level of GBA activity. [Conclusions] We created a novel prodromal mouse model to study the disease pathogenesis and develop novel therapeutics for PD and also revealed the mechanism by which heterozygous *gba* deficiency contributes to PD through abnormal lipid metabolism under conditions of an altered asyn expression *in vivo*.

## AP-02-4 Suppression of Spt4 ortholog reduces expanded SCA36 GGCCUG repeat aggregation and cytotoxicity

○Natsumi Furuta, Setsuki Tsukagoshi, Kimitoshi Hirayanagi, Yoshio Ikeda
Department of Neurology, Gunma University Graduate School of Medicine, Japan

[Objective]A hexanucleotide GGCCTG repeat expansion in intron 1 of the nucleolar protein 56 gene causes spinocerebellar ataxia type 36 (SCA36), which is a relatively pure cerebellar ataxia with progressive motor neuron involvement. To develop potentially useful therapies for SCA36, small chemical compounds and small interfering RNAs (siRNAs) were examined. [Methods]In the present study, SCA36 cell models were generated by introducing the expanded GGCCTG/CAGGCC repeats into the cultured cells. Five candidate small chemical compounds and siRNAs for silencing the transcription elongation factor yeast Spt4/Spt5-ortholog were examined by the assays for RNA foci quantification and cytotoxicity. [Results]Sense $(GGCCUG)_{exp}$ but not antisense $(CAGGCC)_{exp}$ RNA foci were detected in the cells. Glycine-proline dipeptide repeat (DPR) proteins due to repeat-associated non-ATG translation rarely occurred in cells expressing expanded GGCCTG repeats. In contrast, cells harboring expanded *c9orf72* GGGGCC/GGCCCC repeats robustly expressed DPR proteins. Spt4-ortholog knockdown selectively reduced the mRNA expression of expanded $(GGCCUG)_{75}$, leading to decreased RNA foci formation as well as DPR proteins, and ameliorated the cytotoxicity in SCA36 cell models. [Conclusions]Although the underlying mechanisms have yet to be elucidated, it is possible that these treatments target RNA secondary structures and specifically regulate the $(GGCCUG)_{exp}$ mRNA expression. These data provide a basis for developing effective therapeutic strategies for the treatment of SCA36 and other repeat expansion disorders.

## AP-02-5 Analyses of CHCHD2 pathophysiology by human brain, iPSC and Drosophila model

○Aya Ikeda[1], Hong Rui Meng[2], Kenya Nishioka[1], Masashi Takanashi[3], Li Yuanzhe[1], Hiroyo Yoshino[2], Tsuyoshi Inoshita[4], Kahori Shiba[4], Akio Mori[1], Risa Nonaka[5,11], Nana Izawa[1,6], Kei-ichi Ishikawa[1,5], Hidemoto Saiki[7], Masayo Morita[8], Manabu Funayama[1,2,5], Masato Hasegawa[9], Hideyuki Okano[6], Wado Akamatsu[5], Yuzuru Imai[1,10], Nobutaka Hattori[1,2,4,5,10]
[1] Department of Neurology, Juntendo University School of Medicine, Tokyo, Japan, [2] Research Institute for Diseases of Old Age, Graduate School of Medicine, Juntendo University, Tokyo, Japan, [3] Department of Neurology, Juntendo Koshigaya Hospital, Saitama, Japan, [4] Department of Treatment and Research in Multiple Sclerosis and Neuro-intractable Disease, Juntendo University Graduate School of Medicine, Tokyo, Japan, [5] Center for Genomic and Regenerative Medicine, Graduate School of Medicine, Juntendo University, Tokyo, Japan, [6] Department of Physiology Keio University School of Medicine, Tokyo, Japan, [7] Department of Neurology, Tazuke Kofukai Medical Research Institute and Kitano Hospital, Osaka, Japan, [8] Department of Neurology, Jikei University Katsushika Medical Center, Tokyo, Japan, [9] Department of Dementia and Higher Brain Function, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan, [10] Department of Research for Parkinson's Disease, Juntendo University Graduate School of Medicine, Tokyo, Japan, [11] Department of Diagnosis, Prevention and Treatment Dementia, Graduate School of Medicine, Juntendo University, Tokyo, Japan

[Objective]To evaluate the brain pathology of a patient with *CHCHD2*p.T61I and explore the pathophysiological analysis of CHCHD2 protein by using brain tissues, iPS cells (iPSCs), and *Drosophila*model. [Methods]We obtained the brain tissue from a patient with p.T61I in *CHCHD2*, and performed neuropathological and biochemical analyses. iPSCs were established from another patient with p.T61I and differentiated into dopaminergic neurons. *Drosophila*models expressing human wild-type (WT) CHCHD2 or T61I mutant in the presence or absence of alpha-Synuclein (alpha-syn) were characterized in terms of the neuronal phenotypes and neuropathology.[Results]Pathological findings of the brain tissues presented widespread Lewy bodies and neuritis, along with co-localized accumulation of alpha-syn and amyloid-beta, mainly in the limbic area and the neocortex. Western blotting analysis revealed highly aggregated alpha-syn and CHCHD2 in the sarkosyl-insoluble fraction, higher expression of alpha-syn and lower amounts of CHCHD2 in the sarkosyl-soluble fraction in the patient brain. CHCHD2 was co-localized with alpha-syn in Lewy bodies. Similar pathogenic phenotypes were observed in iPSC-derived dopaminergic neurons. *Drosophila*expressing CHCHD2 T61I, but not WT, showed an affliction of motor ability, shorter lifespan and the appearance of alpha-syn aggregation in the brain with age. [Conclusions]Our study suggests that mutations of CHCHD2 produce widespread Lewy body pathology, affecting the protein stability of alpha-syn.

## AP-02-6 Chronic cerebral hypoperfusion accelerates amyloid beta aggregation in APP/PS1 mice model

○Taro Bannai[1], Tatsuo Mano[1], Xigui Chen[2], Ryo Ohtomo[1], Gaku Ohtomo[1], Tadafumi Hashimoto[3], Hitoshi Okazawa[2], Takeshi Iwatsubo[3], Shoji Tsuji[1], Atsushi Iwata[1], Tatsushi Toda[1]
[1] Department of Neurology Graduate School of Medicine, The University of Tokyo, Japan, [2] Department of Neuropathology, Medical Research Institute and Center for Brain Integration Research, Tokyo Medical and Dental University, [3] Department of Neuropathology Graduate School of Medicine, The University of Tokyo

[Objective] To elucidate how chronic cerebral hypoperfusion (CCH) modifies the process of amyloid β (Aβ) deposition. [Methods] Bilateral carotid artery stenosis (BCAS) operation was performed in 10-week-old APP/PS1 transgenic mice and compared with sham operated mice. The mice were subjected to *in vivo* brain microdialysis to assess the dynamics of soluble Aβ in the brain interstitial fluids (ISF) 2 weeks after the operation, and to size exclusion chromatography of the PBS-soluble fraction of brain lysates to separate Aβ oligomers 15 weeks after the operation. To assess the ISF dynamics, we injected fluorescent tracer into the cisterna magna 2 weeks after the operation and examined how the cerebrospinal fluids (CSF) enter and spread from para-arterial space into brain parenchyma. [Results] BCAS-operated mice showed a significant increase of amyloid plaques in both cerebral cortex and hippocampus was detected 30 weeks after the operation (sham; 0.598 ± 0.071%, BCAS; 0.908 ± 0.107%, p=0.0286). Basal Aβ40 and Aβ42 levels of the brain ISF were decreased (sham; 26.27 ± 0.62pM, BCAS; 11.82 ± 0.33pM, p<0.0001), and Aβ oligomers with high molecular size of the PBS-soluble fractions were increased (sham; 24.16 ± 2.775%, BCAS; 38.45 ± 3.581%, p=0.0197) in the BCAS-operated mice. BCAS decreased the speed of the flux of CSF to the para-vascular space and brain parenchyma (p=0.0007; 2-way repeated measures ANOVA). [Conclusions] Our data suggested that CCH attenuate the dynamics of ISF, increase high molecular Aβ oligomers, and promotes soluble Aβ aggregation resulting in enhanced Aβ deposition.

## APe-01-1 Reversal of neuropathic pain by spinal segment-targeted subpial dual gene (GAD65 and VGAT) delivery

○Takahiro Tadokoro[1], Mariana Bravo-hernandez[1], Oleksandr Platoshyn[1], Atsushi Miyanohara[2], Silvia Marsala[1], Martin Marsala[1,3]
[1] Neuroregeneration Laboratory, Department of Anesthesiology, University of California, San Diego, USA, [2] Vector Core Laboratory, University of California, San Diego, [3] Institute of Neurobiology, Slovak Academy Of Sciences, Kosice, Slovakia

**Purpose:** The decrease in GABAergic tone associated with peripheral nerve or spinal cord injury has been postulated to play a role in a development of neuropathic pain (NP). By using a novel spinal subpial gene delivery technique, we studied the anti-nociceptive potency of segment (s)-targeted dorsal horn GAD65 and VGAT genes delivery in mouse NP model. **Method:** i) Adult mice with NP (n=19, partial sciatic nerve ligation) were injected 0.5 μl of a mixed AAV9-GAD65/VGAT vector (s) or vehicle unilaterally into dorsal subpial space of L3-5 segments. To test for anti-nociceptive potency tactile hypersensitivity and brush-evoked nociception were measured. After 12-weeks survival, immunofluorescence staining (IF, n=9), immunofluorescence in situ hybridization (FISH, n=8) and electron microscopy (EM, n=4) were performed. ii) Vgat-ires-Cre and Vglut2-ires-Cre mice with NP received subpial injection of AAV9-Lox-PLox2272-GAD65/VGAT and were tested (n=8). **Result:** i) In contrast to the vehicle-injected mice, the treated mice showed a complete reversal of nociceptive response. IF, FISH and EM showed a potent upregulation of GAD65 and VGAT expression in inhibitory interneurons and appearance of mixed excitatory-inhibitory phenotype in endogenous excitatory interneurons (EN). ii) There was a potent reversal of pain behavior in Vglut2-ires-Cre but not in Vgat-ires-Cre mice. **Conclusion:** The present study shows that subpial delivery of GAD65 and VGAT genes has a potent anti-nociceptive effect and that the primary mechanism associated with this effect is the induction of inhibitory phenotype in EN.

## APe-01-2 Lenticulostriate arteries and basal ganglia changes in CADASIL, a 7.0-T MRI study

○Chen Ling[1], Xiaojing Fang[1], Qinle Kong[2], Zihao Zhang[2], Wei Zhang[1], Zhaoxia Wang[1], Yun Yuan[1]
[1] Department of Neurology Peking University First Hospital, China, [2] State Key Laboratory of Brain and Cognitive Science, Beijing MR Center for Brain Research, Institute of Biophysics, Chinese Academy of Sciences

**Objective:** Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) mainly affects the arterioles.We aimed to analyze the changes of lenticulostriate arteries (LSAs) and basal ganglia in CADASIL by 7.0-T MRI. **Methods:** We performed brain examination in forty-six CADASIL patients (42.85±9.37 years old) and forty-six age-matched controls (41.20±10.08 years old) with a 7.0-T MRI scanner. Then the number of stems and branches of the LSAs as well as the number of lacunar infarctions and microbleeds in basal ganglia were calculated. The correlation between the number of LSAs and the number of lesions in basal ganglia was also analyzed. **Results:** Compared with controls, CADASIL patients had decreased number of branches of LSAs ($P$=0.028) while the number of stems remained unchanged ($P$=0.846). When compared for different age groups, we found that younger CADASIL patients (<45 years old) still had fewer LSAs branches ($P$=0.008), however this difference was abolished in old patients (≧45 years old) ($P$=0.844). In CADASIL patients, lacunar infarctions (86.96% of the patients) were more common than microbleeds (39.13% of the patients) in the basal ganglia, and no correlation was found between the number of branches of LSAs and the number of lacunar infarctions ($\rho$=-0.149, $P$=0.324) or microbleeds ($\rho$=-0.174, $P$=0.246). **Conclusions:** CADASIL patients had fewer number of LSAs branches compared with controls, and the difference was mainly in young subjects. The number of basal ganglia lesions of CADASIL patients was independent of the number of LSAs.

## APe-01-3 A Rapid Molecular Diagnostic Method for Spinal Muscular Atrophy

○Kai Chen Wang[1], Szu Hsien Wang[2], Edward Lee[2], Tai Pei Lin[3], Kuan Chien Chiang[2]
[1] Cheng Hsin General Hospital, Taipei, Taiwan, [2] MBGEN Biosciences, Taipei, Taiwan, [3] Cold Spring Biotech Corp., Taiwan

**Purpose:** Spinal muscular atrophy (SMA) is a common autosomal recessive disorder with an estimated prevalence of 1 in 10,000 live births. The disorder is caused by survival motor neuron 1 gene (SMN1) deficiency leading to dysfunction of limb movement and even dyspnea. A novel method for SMA detection has been used for the compare to the current method. **Method:** A novel method for SMA detection has been developed. Genomic DNA from whole blood, amniotic fluid, or dried blood spots were analyzed by the newly designed SMA Detection Kitin ClarityTM Digital PCR (dPCR) System and MLPA (Multiplex ligation-dependent probe amplification), the current testing golden standard for SMA for determining copy numbers of SMN1 and SMN2 genes. All study subjects signed with the consent agreement form approved by local ethical committee. **Results:** 272 clinical samples were enrolled and used to establish the cut-off ranges for unaffected individual, SMA carrier and SMA patient categories. After setting the cut-off range for each group, we further analyzed 12 samples by both dPCR-based method and MLPA. One hundred percent concordant results were obtained between the two testing methods. **Conclusion:** The newly designed SMA Detection Kit provides a robust and precise approach to distinguish unaffected individuals, SMA carrier and SMA patients. It enables high-throughput screening without sophisticated protocols, low costs but powerful.

## APe-01-4 Aberrant amygdala activation to pain-elicited fear processing in patients with fibromyalgia

○Fu-jung Hsiao[1], Wei-ta Chen[1,2], Shuu-jiun Wang[1,2]
[1] Brain Research Center, National Yang-Ming University, Taiwan, [2] Department of Neurology, Taipei Veterans General Hospital, Taipei, Taiwan

**Objective:** The present study aimed to characterize the altered dynamic activation of the conditioned fear related brain structures in Fibromyalgia (FM) using magnetoencephalogrphic recording. **Methods:** Consecutive patients with FM (aged 20-60 years) and age-matahced healthy controls were enrolled. Delay fear conditioning paradigm was performed and the brain activation was recorded using magnetoencephalography (MEG). The MEG data with individual MRI was further analyzed by distributed deep brain model. Then the regions of interest (ROIs), including the bilateral anterior insula, ACC and amygdala areas, were selected from the standard atlas. The current densities within the ROIs were extracted and examined for the factor of group and the correlation with clinical scores.The hospital's institutional review board approved the study protocol, and each participant provided written informed consent. **Results:** A total of 82 subjects participated in this study, including 30 controls and 52 patients with FM. In healthy control, discernible amplitdue decreases of bilateral amygdala were observed for recording block. The amplitude decreases in the right amygdala area was smaller in FM than in healthy control ($p < 0.001$). In patients with FM, the first-block activation strength were negatively correlated with PSS in the left ($r=-0.472$, $p < 0.001$) and right amygdala areas ($r=-0.413$, $p = 0.002$). **Conclusion:** Alterd amygdala modulation characterized the maladaptive emotional processing in FM. Moreover,the stress level in FM was associated with the amygdala activation.

## APe-01-5 Withdrawn

## APe-01-6 An HCN channel inhibitor benefits a mouse model of spinal muscular atrophy

○Likai Tsai, Chien-lin Chen, Hsing-jung Lai
National Taiwan University Hospital, Taiwan

**Objective** We previously demonstrated that increased hyperpolarization-activated cyclic nucleotide-gated (HCN) current underlies the aberrant excitability of motor axons in spinal muscular atrophy (SMA). This study investigated the effect of HCN inhibitors in a mouse model of SMA. **Methods** Neonatal SMA mice and control littermates were treated with three different HCN inhibitors, including ZD7288, ivabradine, and zatebradine at different dosage via daily intraperitoneal injection (n=16-51 in each group). Mice with or without treatment were subjected to survival analysis, motor functional tests (righting test, hanging test, and tilting test), and pathological studies (spinal cord and neuromuscular junction). The expression of SMN was analyzed using Western blotting. **Results** SMA mice treated with ZD7288 (1 and 2 mg/kg) showed significantly less early mortality and better motor function than untreated SMA control. On the other hand, ivabradine and zatebradine did not benefit SMA mice in lifespan or motor behaviors. Treatment with ZD7288 did not reduce spinal motor neuronal death. However, SMA mice receiving ZD7288 treatment (2 mg/kg) showed significantly higher percentage of innervated neuromuscular junction than untreated SMA mice at postnatal day 8. Notably, ZD7288 treatment did not alter SMN expression in spinal cord of SMA mice. **Conclusions** ZD7288, an HCN channel inhibitor, benefits a mouse model of SMA in early survival and functional behaviors via restoration of neuromuscular junction architectures.