AO-01-1 Monomerization of TDP-43 is a key determinant for inducing TDP-43 pathology in ALS

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[Background] Cytoplasmic aggregation of TDP-43, also known as TDP-43 pathology, is a pathological hallmark of amyotrophic lateral sclerosis (ALS). Recently, N-terminal dimerization of TDP-43 has been reported, but its role in ALS pathogenesis remains unknown. [Methods] We evaluated the dimer/monomer state of TDP-43 in postmortem cerebral cortex from sporadic ALS cases, using disuccinimidyl glutarate (DSG) crosslinking. Biochemical analyses in Neuro2a cells or iPSC-derived motor neurons expressing the dimerization-deficient mutants of TDP-43 were performed. We established "TDP-DiLuc", a novel bimolecular fluorescence complementation assay for the high-throughput evaluation of TDP-43 dimerization in living cells. [Results] DSG crosslinking revealed that the ALS brains had a significant decrease in the dimer/monomer ratio of TDP-43 compared to the controls. Expression of dimerization-deficient TDP-43 in cells recapitulated TDP-43 pathology. We also identified that the nuclear export of monomeric TDP-43 is mediated by Nxf1. Furthermore, TDP-DiLuc revealed that various stresses, including the transcription inhibition linked to aberrant RNA metabolism in ALS, lead to spliceosomal defects and impairment of endogenous TDP-43 dimerization, which preceded the apparition of TDP-43 pathology. [Conclusions] We discovered for the first time that TDP-43 monomerization is a critical determinant for the development of TDP-43 pathology. Monomerization of TDP-43, an early molecular event of TDP-43 pathology, might constitute a potential biomarker and an attractive therapeutic target for ALS.

AO-01-2 Dysregulated endocannabinoid metabolism is a therapeutic target for amyotrophic lateral sclerosis

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[Objective] This study aims to reveal progression factors of sporadic ALS by metabolomics and to develop therapy targeting dysregulated metabolites. [Methods] Subjects with sporadic ALS whose disease durations were less than 2 years and healthy controls (HC) were recruited. Disease severity was evaluated with ALSFRS-R longitudinally. Metabolome analysis on serum of subjects was performed with UPLC-MS/MS. We then selected several chemical compounds which modulate the identified metabolic changes in sporadic ALS and examined the efficacy of them in TDP-43 (A315T) and SOD1 (G93A) cellular models of ALS. Hit chemical compounds were further administered to SOD1 (G93A) transgenic mice. Body weight, rotarod test, survival and pathology of lumber spinal cord were analyzed. [Results] Twenty-six subjects with sporadic ALS and 10 HCs were analyzed. Mean change of ALSFRS-R was -1.1/month. Subjects with ALS were divided into two groups based on the progression rate defined by ALSFRS-R changes. Metabolomics demonstrated several metabolic changes including endocannabinoid metabolism. Several endocannabinoids were increased in rapid progressed ALS. *In vitro* analysis demonstrated several effective chemical compounds including PF-04457845, a fatty acid amide hydrolase which inhibits degradation of endocannabinoids. Oral administration of PF-04457845 extended survival and attenuated motor neuron loss in the mutant SOD1 mice. [Conclusions] Endocannabinoid metabolism is a progression factor of sporadic ALS. PF-04457845, an endocannabinoid activator, is a promising candidate for ALS treatment

AO-01-3 CDP-ribitol prodrug treatment ameliorates ISPD-deficient muscular dystrophy

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[Objective] A group of muscular dystrophy, including Fukuyama-type congenital muscular dystrophy, is caused by defects in ribitol-phosphate modification, which is crucial for the functional maturation of α-dystroglycan (α-DG). Currently, no effective treatments are available for this disease group. *Isoprenoid synthase domain containing* (*ISPD*) encodes an enzyme that synthesizes CDP-ribitol, a donor substrate for ribitol-phosphate modification, and its defects are associated with congenital and limb-girdle muscular dystrophies. To explore therapeutic strategies, we established a mouse model and examined gene therapy and prodrug treatment. [Methods] We generated skeletal muscle-selective *Ispd* conditional knockout (cKO) mice and investigated adeno-associated virus (AAV)-mediated gene replacement and CDP-ribitol supplementation. [Results] *Ispd* cKO mice showed reduction in CDP-ribitol levels, abnormal glycosylation of α-DG, and severe muscular dystrophy. AAV gene replacement restored CDP-ribitol levels and rescued the ISPD-deficient pathology. Administration of tetraacetylated CDP-ribitol, which we developed as a prodrug, ameliorated the dystrophic pathology in the cKO mice and rescued abnormal α-DG glycosylation in patient fibroblasts. [Conclusions] We demonstrate that prodrug treatments can ameliorate muscular dystrophy caused by defects in *ISPD*. Our findings provide proof-of-concept for supplementation therapy with CDP-ribitol and could accelerate the development of therapeutic agents for muscular dystrophy and other neuromuscular diseases caused by glycosylation defects.

AO-01-4 Intrinsic blood-brain barrier dysfunction contributes to multiple sclerosis pathogenesis

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[Objective] The mechanisms leading to blood-brain barrier (BBB) dysfunction in multiple sclerosis (MS) are incompletely understood and generally thought to be a consequence of neuroinflammation. Here, we have challenged this view and asked if intrinsic alterations in BBB of MS patients could contribute to MS pathogenesis. [Methods] We made use of human induced pluripotent stem cells (hiPSCs) derived from 6 clones from 3 healthy controls (HC) and 7 clones from 4 MS patients and differentiated them into brain microvascular endothelial cell (BMEC)-like cells as in vitro model of the BBB. [Results] MS-derived BMEC-like cells showed impaired junctional integrity, barrier properties and efflux pump activity when compared to HC-derived BMEC-like cells. Also, MS-derived BMEC-like cells displayed an inflammatory phenotype with increased adhesion molecule expression and immune cell interactions. Moreover, pre-activation of Wnt/β-catenin signaling, known to be involved in BBB maturation and maintenance, in MS-derived BMEC-like cells enhanced barrier characteristics and reduced the inflammatory phenotype. [Conclusions] Our study provides evidence for 1) BBB alteration in MS can be modeled with hiPSC derived BMEC-like cells in vitro, 2) BBB alteration is not the consequence of neuroinflammation but actively involved in MS pathogenesis. Human iPSC-derived BMEC-like cells are thus suitable to explore the molecular underpinnings of BBB dysfunction in MS and assist in the identification of potential novel therapeutic targets for BBB stabilization.

AO-01-5 Decrease of GM1 ganglioside and LAMP2 in PARK24-linked prosaposin gene mutation

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[Objective] Recently, we identified the prosaposin gene (*PSAP*) as a novel gene for familial Parkinson's disease (PD). Four sphingolipid activator proteins (saposins) derived from PSAP activate lysosomal glycosphingolipid degradation. However, it remains unclear how PD-linked *PSAP* mutations could affect the lipid metabolome pathway. [Method] We generated isogenic induced pluripotent stem cells (iPSCs) by introducing the PSAP p.C412Y heterozygous (Het) or homozygous (Ho) mutation using CRISPR/Cas-9 genome-editing technique. The three cell iPSC lines such as wild-type (WT), Het, and Ho were differentiated into dopaminergic neurons (iPSC-mDA), which were applied for lipid analyses. Lysosomal proteins and alpha-synuclein (aS) were also analyzed. Statistical analyses were performed using ANOVA with multiple comparisons. [Results] Unbiased lipidomics revealed that the levels of GM1 ganglioside were significantly decreased by about 70% in Ho while about 45% in Het compared with wild-type iPSCs-mDA ($p < 0.0001$). Immunoblot revealed that the levels of lysosome-associated membrane protein-2 (LAMP2) were significantly decreased in iPSCs-mDA with Ho and Het mutations ($p < 0.001$). Furthermore, the levels of aS in triton X-100 insoluble fraction were significantly increased in iPSCs-mDA with Ho and Het mutations ($p < 0.05$). [Conclusion] Our results suggest a pathological association among PSAP, GM1, and LAMP2 in the pathogenesis of PD, although further studies will be needed.

AO-01-6 IL-6 deposition in the dorsal root of the spinal nerve in Neuromyelitis Optica Spectrum Disorder

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Objective: In neuromyelitis optica spectrum disorder (NMOSD), serious pain often persists as a sequelae of myelitis. IL-6 is a pro-inflammatory cytokine that has been clinically and experimentally implicated in neuropathic pain, and are elevated in the CSF during the acute phase of NMOSD. The purpose of this study is to clarify the pathological relationship between IL-6 deposition and astrocyte damage in the dorsal root of the spinal nerve in patients with NMOSD. Methods: We investigated autopsied tissues of the spinal cord derived from 18 patients with NMOSD. We examined staining patterns of IL-6 at the dorsal root of spinal nerve in the acute, subacute and chronic stages of astrocytopathic lesions using immunohistochemical techniques. Results: The cohort of NMOSD in the analysis comprised 13 women and 5 men. The median age at onset was 54.5 years (range 14-79), and the disease duration was 22.5 months (range 0.6-324). IL-6 deposition at the dorsal root was found in the 11/13 (85%) tissues with acute stage of astrocytopathic lesions, but not in the other stages (subacute lesion: 0/3, chronic lesion: 0/12). Interestingly, the deposition of IL-6 was observed mainly in the transitional zone, where the CNS structures partially protrude into the peripheral nerve. At the site of IL-6 deposition, most lesions showed disruption and loss of astrocytes, with surrounding degenerated and hypertrophic astrocytes. Conclusions: IL-6 was deposited at a high rate in the dorsal root of spinal nerve with astrocyte degeneration, which may relate to the generation of neuropathic pain in NMOSD.

AO-02-1 Identification of seral disease-specific alpha-synuclein seeds using IP-RT-QuIC

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Objective Parkinson's disease (PD) and multiple system atrophy (MSA) are synucleinopathies caused by abnormal alpha-synuclein (aSyn) aggregation. The pathogenic β-sheet seed conformation of aSyn can be found in various tissues, but its potential as a serum biomarker is unclear. METHODS We developed a novel assay system, immunoprecipitation-based real-time quaking-induced conversion (IP/RT-QuIC), to detect aSyn seeds in serum of patients with synucleinopathies. Diagnostic performance was assessed with receiver operating characteristic curve analyses. The morphological characteristics of the IP/RT-QuIC products were evaluated using transmission electron microscopy (TEM), and the seeding properties in cell and mouse models were evaluated. RESULTS We collected sera from 260 participants with synucleinopthies (221 with PD and 39 with MSA) and 128 controls. IP/RT-QuIC displayed high diagnostic performance for the differentiation of the PD versus controls (area under the curve [AUC] 0.95 [95% CI 0.92-0.98]) and MSA versus controls ([AUC] 0.64 [95% CI 0.49-0.79]). TEM analysis revealed that the IP/RT-QuIC-derived fibrils had distinct fibrillar structures for PD and MSA. The propagative potential of the amplified aSyn fibrils was confirmed both in cells and *in vivo*. CONCLUSIONS IP/RT-QuIC enables to detect serum pathogenic aSyn seeds and quite useful diagnostic biomarker for synuclienopathy. Furthermore, the amplified aSyn seeds maintain their disease-specific morphological and propagative properties and are distinguishable between PD and MSA.

AO-02-2 Exosomal microRNA profiles in peripheral blood are useful for early diagnosis of Alzheimer's disease

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Objective:Blood biomarkers to diagnose Alzheimer's disease (AD) are increasingly important. We aimed to identify novel blood exosomal microRNA (miR) markers for AD using human β amyloid precursor protein knock-in (APP-KI) AD model mice and validate them in AD patients. Methods:Plasma exosomal RNA was extracted using an ExoQuick Exosome Isolation and RNA Purification kit (SBI, CA, USA)) from 6-month-old APP-KI (APP^{NL-G-F}) and wild type mice, and 10 human subjects [5 AD patients and 5 age-matched healthy controls (HC)]. MiR varied in APP-KI mice compared to wild type mice was studied by next generation sequencing (Illumina HiSeq), and each candidate miR was validated by individual qPCR assays using the TaqMan microRNA assays (Thermo Fisher Scientific). Each miR amount was normalized to cel-miR-39. Relative expression levels of miR were calculated using the 2-Δ ΔCt method. Results:In APP-KI AD mice, 1 upregulated and 5 downregulated miR were found at the emergence of memory impairment compared to wild type mice. The miR-203 upregulated in AD mice was also >6-fold higher in AD patients than HC ($p < 0.05$) while the most down-regulated miR-30c-2 in AD mice tended to decrease in AD patients compared to HC. Conclusion:In this study, we identified 6 miR as potential blood biomarkers for early AD based on AD mouse results. As upregulation of miR-203 was also confirmed in AD patients, blood exosomal miR-203, a suppressor of cell proliferation and migration, may be useful for early diagnosis of AD in human. Validation using a large scale cohort of demented patients is now under way.

AO-02-3 Dysbiosis in the Salivary Microbiome; Promising Biomarker for Early Detection of Multiple Sclerosis

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Objective: The aim of this study is to reveal the characteristics of salivary microbiome in patients with MS and evaluate its validity as a diagnostic biomarker. Method: We comparatively analyzed salivary microbiome of 59 RRMS patients, 12 SPMS patients, 20 atypical MS patients, 20 neuromyelitis optica spectrum disorder patients, and 60 healthy controls (HC) by 16S rRNA gene data from saliva samples. Results: There were large differences in the composition of salivary microbiome between each patient group and the HC group ($p < 0.001$). Changes in some microbial data had significant association with disease activity of the patients. Then we compared the potentials of diagnostic biomarker between the salivary and fecal microbiome in the exactly matched RRMS and HC cohorts by combining the species or genera selected by the random forest algorithm in machine learning, followed by confirmation with 10-fold cross-validation. We ascertained that the area under the curve (AUC) value distinguishing RRMS from HC based on the saliva data was 0.94 in a discovery cohort and 0.83 in a validation cohort, which markedly surpassed the corresponding AUC values based on the feces data. Even in the comparison between HC and RRMS (disease duration ≦ 5 years) or HC and RRMS (EDSS score ≦1), the AUC value based on the saliva data remained 0.84 and 0.88, respectively. Conclusion: We revealed the dysbiosis of salivary microbiome in various clinical phenotypes of MS, which might be effective for early detection of this disease.

AO-02-4 Altered brain energy metabolism related to astrocytes in tauopathies

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[Objective] In Alzheimer's disease (AD) and progressive supranuclear palsy (PSP), decreased fluorodeoxyglucose (FDG) uptake in the cingulate cortex is considered a marker of neurodegeneration; however, its underlying pathogenesis remains unknown. It is hypothesized that lactate, a glucose metabolite, is produced in astrocytes and subsequently shuttled to neurons as an energy substrate. The current study aims to examine alterations in lactate and glucose concentrations and their association with astrocytic activities in the cingulate cortex of patients with AD and PSP. [Methods] Twenty-five AD, 25 PSP, and 26 healthy control (HC) subjects were enrolled. Lactate, glucose, and myoinositol (mI), an astroglial marker, in the anterior and posterior cingulate cortex (ACC and PCC) were measured by magnetic resonance spectroscopy (MRS). Tau depositions were assessed by PET with ^{18}F-PM-PBB3. [Results] All the patients with AD and PSP were confirmed to have typical distribution of tau pathology. Lactate, glucose, and mI levels were higher in the ACC in PSP and AD, and PCC in AD compared to HC ($p < 0.05$). In these regions, lactate levels correlated positively with mI levels ($p < 0.05$). Lactate levels in the PCC in AD showed a positive correlation with the CDR sum-of-boxes score ($p < 0.05$). [Conclusions] We found elevated lactate and glucose levels accompanied by an increased astroglial marker in the cingulate cortex in AD and PSP. It suggested that the impaired lactate shuttle of reactivated astrocytes disrupts energy utilization, resulting in surplus levels of lactate and glucose.

AO-02-5 A nationwide epidemiological survey of Facial Onset Sensory Motor Neuronopathy (FOSMN) in Japan

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<Objective> FOSMN is a rare disease with only about 100 cases reported worldwide. Cardinal features are initial asymmetrical facial paresthesia and/or sensory deficits followed by bulbar symptoms and spreading of sensory and motor deficits from the face to the scalp, neck, upper trunk, and upper extremities. Its epidemiology and etiology remain unknown. To investigate the prevalence of FOSMN in Japan and establish the characteristics of this disease, we conducted a nationwide epidemiological survey. <Methods> We performed the survey of FOSMN supported by the Ministry of Health, Labour and Welfare, Japan. A questionnaire on FOSMN was mailed to 1,214 randomly selected neurology facilities in Japan. The primary survey asked whether each facility had experienced FOSMN cases in 2019. Facilities with the experience were sent a second questionnaire, asking details on disease characteristics, neurological findings, clinical courses, laboratory findings and treatments. <Results> In the primary survey, we received answers from 604 facilities (49.8%), leading to an estimated number of 41.2 FOSMN cases in Japan. The secondary survey collected details on 21 cases. Dysphagia developed in 95.2% of the cases, of which half had oral phase dysphagia. Treatment was performed in 81%. All received IVIg and 35.3% saw improvement. There were 6 cases with response to treatment, and serum antibody was positive in 4 of them. <Conclusions> This is the first nationwide epidemiological survey of FOSMN in the world and we revealed the clinical features of FOSMN including treatment response in Japan.

AO-02-6 Clinical features and mechanism of LGI4-IgG4-positive inflammatory demyelinating polyneuropathy

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Objective: We reported a novel IgG4 antibodies to Leucine Rich Repeat LGI Family Member 4 (LGI4) in chronic inflammatory demyelinating polyneuropathy (CIDP). We aimed to elucidate clinical features and mechanism of anti-LGI4 antibody-positive ($LGI4^+$) CIDP. Methods: We found 5 $LGI4^+$ CIDP cases in 114 CIDP patients seronegative for neurofascin 155 and contactin-1, and retrospectively surveyed clinical records. The effect of an $LGI4^+$ CIDP patient serum on expression of *Krox20* and *Periaxin*, which independently control myelination, was examined in rat Schwann cells. Results: These 5 patients had a relatively old onset age (mean 56 years, range 42-76), presenting typical CIDP in 4 and multifocal acquired demyelinating sensory and motor neuropathy in 1. The onset was subacute in 3 typical CIDP and chronic in other 2. All showed motor weakness and deep and superficial sensory impairment. Romberg sign and finger tremor were seen in 3. Cerebrospinal fluid examined in 4 cases showed extremely high protein amounts (mean 335 mg/dl, 182 to 541). Intravenous immunoglobulin was effective in 3/3 patients administered. Schwann cells, which we confirmed to express ADAM22, a receptor of LGI4, showed significantly decreased *Krox20* but not *Periaxin* mRNA upon treatment with LGI4-IgG compared with control IgG. Conclusion: $LGI4^+$ CIDP often shows subacute motor and sensory polyneuropathy masquerading Guillain-Barré syndrome. LGI4-IgG4 down-regulates *Krox20* expression by blocking autocrine interaction between secreted LGI4 and ADAM22 in Schwann cells, which may lead to demyelination in this condition.

AP-01-1 AI-based live-cell-image analysis for spinal and bulbar muscular atrophy pathology

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[Objective] Spinal and bulbar muscular atrophy (SBMA)is a neuromuscular disease caused by CAG repeat expansions in the androgen receptor gene. We demonstrate an innovative approach of an AI (artificial intelligence) -based cell-morphologic analysis to determine pathological processes and to find novel therapeutics for SBMA. [Methods] We used a muscular C2C12 cell model of SBMA (97Q cells)and control (24Q cells). We administered 5 alpha-dihydrotestosterone (DHT)which is known to aggravate the pathogenesis of SBMA, and pioglitazone (PG)which is reported to increase the viability of 97Q cells, and evaluated whether the image analysis could reproduce the effect of the drugs.We performed gene expression analysis to identify genes that were dysregulated in 97Q cells. Based on the results, we selected drugs that target the signaling pathway associated with the identified genes. We applied these compounds to 97Q cells and performed the image analysis. [Results] The clustering of DHT-treated 97Q cells moved away from that of 24Q cells. In contrast, the clustering of PG-treated 97Q cells shifted toward that of 24Q cells. From gene expression analysis, we selected naratriptan (NRT), p38 inhibitor, NFkB inhibitor, N-acetylcystein and nifedipine. NRT-treated 97Q cells shifted toward 24Q cells. Nifedipine-treated 97Q cells had no change. As for others, some compounds shifted the morphological phenotype of 97Q cells toward that of 24Q cells.[Conclusions] We develop an AI platform for evaluating living cells to determine the efficacy of drugs, and potentially for further drug screening.

AP-01-2 Two novel variants in CHCHD2 associate with TDP-43 pathology among amyotrophic lateral sclerosis

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[Objectives] The purpose of this study was to analyze the functions of pathogenic variants of CHCHD2 among patients with ALS.[Methods] We implemented the genetic sequencing for CHCHD2 among 944 patients with ALS. Histochemical analysis was performed using the frontal lobe of brain tissues harboring ALS-associated CHCHD2 variants. We generated CHCHD2 knockout SH-SY5Y cells and reintroduced genes for wild-type CHCHD2, ALS-associated P14L, or Parkinson's disease (PD)-associated T61I into these cells. *Drosophila* models expressing CHCHD2 and its ALS- or PD-associated variants were also characterized. [Results] We identified two novel variants of CHCHD2, 8T>G and P14L, among ALS patients. Histochemical analysis revealed TDP-43 proteinopathy in two probands. We observed both CHCHD2 and its paralogue CHCHD10 were reduced in the brain with the P14L variant. Moreover, CHCHD2 P14L, but not T61I, tended to mislocalize in the cytosol of SH-SY5Y cells. Similar to the results in mammalian cultured cells, CHCHD2 P14L was dissociated from mitochondria in *Drosophila*. Expression of CHCHD2 P14L caused the reduction in mitochondrial Ca^{2+} uptake and the increase in cytosolic Ca^{2+} flow in *Drosophila* neuron terminals. [Conclusions] ALS patients with CHCHD2 variants exhibited TDP-43 pathology. The mitochondrial dissociation of CHCHD2 by P14L variant might affect the Ca^{2+} homeostasis in neurons, which is buffered by mitochondria. Dysregulation of Ca^{2+} homeostasis leads to excitotoxicity of motor neurons, which has been reported as a pathomechanism of ALS and a cause of TDP-43 proteinopathy.

AP-01-3 Discovery of RNA-binding proteins as modifiers that distinguish the SCA36 and C9-ALS pathomechanisms

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OBJECTIVE: Spinocerebellar Ataxia type 36 (SCA36)is caused by an abnormal expansion of GGCCTG repeat (>25)in the first intron of *NOP56* gene, which is one type of non-coding repeat expansion disorders (NRDs)like as *C9orf72*-linked ALS (C9-ALS). A structure called RNA foci, which is an aggregation of GGCCTG repeat RNAs, and dipeptide repeat protein (DPR)produced by unconventional repeat associated non-ATG (RAN)translation are observed in SCA36 patients. However, the pathomechanisms of SCA36 and the difference from the C9-ALS pathomechanism are still unknown. METHODS: To elucidate the pathomechanism, we established novel *Drosophila* as SCA36 genetic models, which have striking advantages of rapid generation cycle. RESULTS: We found that expression of expanded GGCCTG repeats in the compound eyes caused obvious eye degeneration, while normal-length GGCCTG repeats did not. Consistent with SCA36 patients, intranuclear RNA foci were detected. Furthermore, we detected the expression of poly (Gly-Pro), which is produced by RAN translation. We have identified RNA-binding protein X (RBPX)that suppresses compound eye degeneration and decreases poly (Gly-Pro)expression. Moreover, we also identified RBPY that enhanced the eye degeneration in SCA36 flies, which otherwise suppresses the eye degeneration in C9-ALS model flies expressing GGGGCC repeat RNA. CONCLUSIONS: We established the first genetic model of SCA36. Further studies using this model will accelerate understanding pathogenesis and developing therapy of SCA36 and other NRDs including C9-ALS.

AP-01-4 Late-onset multiple system atrophy in the brain resource center

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[Objective] The onset of multiple system atrophy (MSA)is usually during 50-60 years of age; onset after 75 years is indicated as a feature not supporting the diagnosis of MSA on the current criteria. This study reviewed the characteristics of late-onset multiple system atrophy (LOMSA). [Methods] We defined LOMSA as MSA that develops after the age of 75 years. Disease onset was set as awareness of either motor or autonomic symptoms. We reviewed the clinical and neuropathological findings of 202 pathologically confirmed patients with MSA, who were consecutively autopsied between 1978 and 2020 at our brain resource center. Thirty-two patients were excluded due to insufficient data. [Results] Mean age of onset for the 170 patients included in the study was 60.2 ± 9.3 years (range, 33-84 years). Twelve of the 170 patients (7.1%)were identified as LOMSA. The 12 patients with LOMSA showed a shorter disease duration (4.0 ± 1.6 years)than the other 158 (7.6 ± 4.2 years). Pathologically, 8 of 12 patients with LOMSA were classified as the striatonigral degeneration (SND)dominant type. In contrast, the other four patients exhibited the olivopontocerebellar atrophy (OPCA)dominant type and the OPCA-SND mixed type. [Conclusions] LOMSA accounted for 7.1% of our cohort. Thus, a late-onset presentation does not rule out MSA. Our study suggests that the second consensus statement for the diagnosis of MSA needs to be revised regarding the onset age range of MSA.

AP-01-5 Humanized-AQP4 rat NMO model develops severe astrocytopathy by patient-derived NMO-IgG

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[Objective] So far, several animal models of neuromyelitis optica (NMO)have been developed, but these models using NMO-patient-derived IgG (NMO-IgG)showed quite mild astrocytic damage. It is considered that one of the reasons is low affinity of NMO-IgG against rodent AQP4. The purpose of this study is to reproduce more severe astrocytic lesions transferring NMO-IgG to humanized-AQP4 (hAQP4)rats. [Method] We generated hAQP4 rats by genome editing technology. We immunized 5 hAQP4 and 3 wild-type rats (8-10 weeks old)with myelin basic protein conjugated with complete Freund adjuvand. When rats showed initial signs of tail paralysis or body weight loss, we intraperitoneally injected 20mg or 40mg of NMO-IgG, or 40mg of control-IgG. 2 days after antibody injection, we sacrificed rats and examined histopathology of brains and spinal cords and compared the AQP4 loss area in the spinal cord between hAQP4 and wild-type rats. [Result] AQP4 loss lesions were found only in NMO-IgG group. These lesions were associated with GFAP loss and neutrophil infiltration, while axons and myelin sheaths were preserved. The location of the lesions was mainly in the peri-third ventricle, medulla oblongata and spinal cord. The proportion of AQP4 loss lesion in spinal cord was significantly higher in the group receiving 20mg or 40mg of NMO-IgG in hAQP4 rats than those in wild-type rats (20mg: 7.0 ± 3.9% vs 4.1 ± 2.0%, $p<0.05$, 40mg: 11.0 ± 3.9% vs 4.5 ± 1.9%, $p<0.05$). [Conclusion] By using hAQP4 rat, NMO-IgG could produce more diffuse astrocytopathic lesions, which is compatible with human NMO pathology.

AP-01-6 SNCA p.V15A, a novel pathogenic variant for familial Parkinson's disease

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[Background] *SNCA* is known as a pathogenic gene for familial Parkinson's disease (PD). It encodes a-Synuclein (a-Syn), which is the main component of Lewy bodies. [Methods] A genetic screening for *SNCA* mutations from 964 PD subjects and 324 controls was performed. Using the identified variant, its effects on binding to phospholipid membrane, self-aggregation, and seed-dependent aggregation in cultured cells were examined. [Result] Genetic screening identified *SNCA* p.V15A from two families (Family A and B)with PD, none from controls. p.V15A was extremely rare in several public databases and predicted as pathogenic in *in silico* tools. The proband of Family A developed PD at 42, presented levodopa-induced dyskinesia (LID)at 52. She started using a wheelchair at 57 along with mild cognitive decline. Her younger sibling developed PD at 48 and gradually suffered from LID. Their cousin also developed PD. Mother of the proband was unaffected carrier at 85. In Family B, the proband was diagnosed as Dementia with Lewy body. His father was also PD. Replacement of V15 with A in a-Syn protein showed decreased affinity to phospholipid membrane, increased aggregation property. Moreover, a-Syn V15A promoted seed-dependent a-Syn aggregation in cultured cells. The appearance of a-Syn V15A fibrils was almost similar to that of WT a-Syn fibrils. [Conclusion] *SNCA* p.V15A seems to be a novel pathogenic variant for PD from clinical data. V15A a-Syn weakened the binding to phospholipid membrane and promoted a-Syn aggregation, showing intermediate properties between WT and known pathogenic mutants.

AP-02-1 Toxic Aβ_{42} conformer may accelerate the onset of Alzheimer's disease in the preclinical stage

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Background: Alzheimer's disease (AD)is characterized by pathological aggregates of amyloid β-protein (Aβ)and tau protein, senile plaques, and neurofibrillary tangles. Toxic Aβ conformers play an important role in the progression of AD. The ratio of the toxic conformer to total Aβ_{42} in cerebrospinal fluid (CSF)was significantly high in AD and mild cognitive impairment (MCI)due to AD using an enzyme-linked immunosorbent assay kit with a 24B3 antibody. 24B3 is an antibody highly specific to the conformer with a turn at positions 22/23 of Aβ_{42} (toxic turn). Objective: We compared the toxic Aβ conformer at different stages of AD to identify its contribution to AD pathogenesis. Methods: We compared 5 patients with preclinical AD, 11 patients with MCI due to AD, 21 patients with AD, and 5 healthy controls to measure CSF levels of total Aβ_{42}, total tau, tau phosphorylated at threonine 181 (p-tau), and toxic Aβ conformers. All were classified using the Clinical Dementia Rating. Cognitive function was assessed using the Japanese version of the Mini-Mental State Examination (MMSE-J). Results: Toxic Aβ conformer level was insignificant between groups, but its ratio to Aβ_{42} was significantly higher in AD than in preclinical AD ($p < 0.05$). Toxic Aβ conformer correlated positively with p-tau ($r = 0.67$, $p < 0.01$)and p-tau correlated negatively with MMSE-J ($r = -0.38$, $p < 0.05$). Conclusion: Toxic Aβ conformer may trigger tau accumulation leading to neuronal impairment in AD pathogenesis.

AP-02-2 Association between abnormal blood pressure fluctuations and visual hallucination in PD/DLB

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【目的】幻視を発症したPD患者は、未発症のPD患者と比較して血圧変動が大きく、交感神経節後線維の障害が強いとの報告がある。また、何もないところに動物や人が見える幻視（formed visual hallucination：fVH）を発症したPD/DLBの剖検脳では、海馬・扁桃体に有意に高度のLewy小体の蓄積がみられており、これらを含む辺縁系からは、自律神経節後神経細胞に至る複数のシナプスを介した連絡があり、辺縁系は精神症状のみならず自律神経活動にも関与している。本研究では異常な血圧変動がPD/DLBのfVHと関連するかを明らかにし、MRIによる内側側頭葉萎縮との関連を検討した。【方法】症例・対照研究。対象はPD/DLB患者の合計94名（PD 94%、男性51%、年齢72.6±8.5歳（平均±SD）、罹病期間 8.5±5.7年、H-Y 3（中央値）。自由行動下血圧測定により日中の収縮期血圧標準偏差（SBP-SD）、血圧日内変動指標（dipper, riser type）を同定した。また、頭部MRI画像をVSRAD advance®を用いて関心領域（海馬、扁桃体、嗅内野の大部分）の萎縮の程度を測定した。fVHと血圧変動の関連、内側側頭葉萎縮との関連には多変量ロジスティック解析を用いて検討した。【結果】対象はfVHを発症したPD/DLB群（39例）とfVH未発症のPD/DLB群（55例）。fVHはSBP-SDの増大と有意に関連し（OR=1.6, 95%CI 1.1-2.3）、riser typeとも有意に関連していた（OR=2.9, 95%CI 1.1-8.0）（いずれも、年齢、性別、H-Y、罹病期間、LED、認知機能で調整）。fVH群は内側側頭葉の関心領域が広範に萎縮し（OR=1.3, 95%CI=0.99-1.6）（性別、年齢、H-Y、罹病期間で調整）、内側側頭葉の萎縮はriser typeと有意に関連していた。【結論】PD/DLBにおけるfVHの発症は血圧変動の増大、異常な血圧日内変動（riser type）と関連があり、また内側側頭葉の萎縮はfVHとriser typeの双方に関連していた。辺縁系における神経変性が、幻視と異常な血圧日内変動の両者の原因となっている可能性が示唆された。

AP-02-3 Molecular epidemiology of degenerative ataxias in Japan based on J-CAT study

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[Objective] The aim of the study was to investigate the molecular epidemiology of degenerative ataxias in Japan. [Methods] From December 2016 to November 2021, 2043 patients were registered in Japan Consortium of Ataxias (J-CAT)nation-wide, roughly proportional to prefectural population density. Initial mutational analysis including PCR fragment analysis and repeat-primed PCR of SCA1, SCA2, MLD/SCA3, SCA6, SCA7, SCA8, SCA12, SCA17, SCA31, SCA36 and DRPLA were conducted for 1550 patients with informed consent. Whole exome/genome sequencing (WES/WGS)was conducted for 313 mutation-negative patients, prioritized for familial cases or sporadic cases with younger onset, whose pathogenic variants were confirmed by Sanger sequencing method. [Results] Initial mutational analysis established the diagnosis in 712 patients (46.0%)including 229 with SCA31, 205 with SCA6, 126 with MJD/SCA3, 52 with DRPLA, 28 with SCA2, 24 with SCA1, 14 with SCA8, 12 with MJD (intermediate), 9 with HD and 9 with SCA 36. WES/WGS also established the diagnosis in 20 patients including 6 with SCAR8 (*SYNE1*), 3 with SCA42 (*CACNA1G*), 2 with Perrault syndrome 1 (*HSD17B4*), 2 with HSANIID (*SCN9A*), 2 with Gordon holmes syndrome (*RNF216*), 1 with DEE32 (*KCNA2*), 1 with EA2 (*CACNA1A*), 1 with GM2-gangliosidosis (*HEXA*), 1 with Hartnup disorder (*SLC6A19*), and 1 with hypotonia, ataxia, developmental delay, and tooth enamel defect syndrome (*CTBP1*). Further confirmation of variants is under way. [Conclusions] J-CAT has elucidated the most updated feature of molecular epidemiology of degenerative ataxias in Japan.

AP-02-4 Clinicopathological findings of anti-mitochondrial antibody associated myositis

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【目的】抗ミトコンドリアM2抗体（Anti-mitochondrial M2 antibody：AM2A）が陽性となる筋炎の存在が報告されてきているが，それが独立した筋炎の一亜型であるかどうかは不明である．本研究の目的はAM2A陽性例の臨床病理学的特徴を明らかにすることである．【方法】2008年から2020年の13年間に当センターで筋病理診断を実施したAM2A陽性245例のうち，アーチファクトのため組織学的評価が困難な9例を除外した，計236例を対象とし筋病理学的評価を行った．1）壊死線維または再生線維が存在する，2）筋線維にHLA-ABCが発現している，3）CD8陽性細胞が20倍視野において20個以下，4）筋線維でミクソウイルス耐性タンパク質Aが発現していない，の4項目を満たす場合，免疫介在性壊死性ミオパチーと分類した．併せて，病歴情報の解析も行った．【結果】155例（65.6%）が免疫介在性壊死性ミオパチーに分類された．一方，筋病理所見，自己抗体検査，遺伝学的検査の結果から別の神経筋疾患と診断された例は29例（封入体筋炎9例，神経原性疾患5例，皮膚筋炎3例，抗合成酵素症候群3例，他），非特異的な病理を呈する例は52例であった．全体のうち肉芽腫を認めた例は7例（2.9%）であった．免疫介在性壊死性ミオパチー155例のうち80例で筋線維膜上への膜侵襲複合体の沈着を認めた．1年以上の慢性経過をたどる症例が104/143例（72.7%）存在し，不整脈の合併頻度は75/144例（52%）と高値であった．【結論】本研究はこれまでに報告された中で世界最大規模のAM2A陽性筋炎のコホートである．免疫介在性壊死性ミオパチーの病理を呈するAM2A陽性筋炎が全体の約2/3を占めた．

AP-02-5 Efficacy and safety of mexiletine hydrochloride in spinal and bulbar muscular atrophy

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Objective: Patients with spinal and bulbar muscular atrophy (SBMA)often experience muscular weakness or myotonia-like muscle contraction during cold exposure. These myotonic syndromes are caused by muscle membrane hyperexcitability which are led to excessive sodium current in skeletal muscle. The aim of this study is to explore the the efficacy and safety of mexiletine in SBMA patients. Methods: We conducted a randomized, double-blind, placebo-controlled, 2 × 2 crossover, phase II trial at 3 centers. Twenty participants took 100 mg of mexiletine or lactose, three times a day for 4 weeks. The primary endpoint of this trial was the change in the difference in distal latencies under room temperature and cold exposure conditions. Secondary outcome measures included the quantitative muscle strength in the upper limbs, tongue pressure, respiratory function test, and timed walk test. We also evaluated the longitudinal change in ALSFRS-R. Results: There was no statistically difference in the primary endpoint. However, mexiletine improved tongue pressure and 10-s grip and release test under cold exposure. Furthermore, ALSFRS-R which reflect comprehensive motor function in SBMA patients was tend to be improved in the mexiletine group (treatment effect size; 0.51 (-0.10 to 1.12), $p = 0.094$). In addition, quantitative motor function tests tended to improve in the mexiletine group. There was no serious adverse event throughout the clinical trial period. Conclusion: Voltage-gated sodium channel blockers could be effective in improving overall motor function in SBMA patients.

AP-02-6 Correlation between serum α-synuclein aggregation and proteomics in Parkinson's disease

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Objective: In Parkinson's disease (PD), it has been proved that alpha-synuclein (AS)aggregates are present not only in the central nervous system but also in the blood, retina, skin, digestive tract and other parts of the body. However, the molecular hallmark of pathological aggregation factors in PD are unclear. To elucidate factors affecting aggregation of AS, we performed real-time quaking-induced conversion (RT-QuIC)and proteomics of plasma from PD and analyzed the association between AS aggregation rate and data of proteomics. Methods: The study was included 86 PD patients (44 females, 22 males, age 66.1 ± 8.8 years, Hoehn & Yahr stage; HY 1.96 ± 0.76). An RT-QuIC method was performed using the serum to measure the forming rate, T1 / 2, Tmax, and AUC of abnormally structured AS. The proteomics analysis was performed using the plasma in the patients. Results: There was a positive correlation between the forming rate of AS aggregates and cathepsin D ($r = 0.237$)and TNFRSF13C ($r = 0.235$), whereas it had a negative correlation with HO-1 ($r = -0.377$). In addition, TLT-2 ($r = -0.241$)and VEGFR-2 ($r = -0.243$)were negatively correlated with UPDRS-III and HY, respectively. Conclusions: The forming rate of AS aggregates in the serum of PD patients is associated with lysosomal function, maintenance of B cell, and oxidative stress. Furthermore, clinical symptoms are associated with the function of T cell and VEGF which has neuroprotective effect. Thus, the alterations of lysosomal function, immune system and redox status might influence on the pathological aggregation of AS.

APe-01-1 Screening compounds library using seed-dependent cellular tau aggregation for Alzheimer's disease.

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Objective: While in previous studies, in vitro tau protein aggregation has been used for screening drug library to reposition a drug, in our study, Alzheimer's Disease (AD) tau-seeded cellular model was used for screening. Methods: SH-SY5Y cells were transfected with Tau-CTF24 (243-441 aa), and seeded with AD patient's brain seed to induce intracellular tau aggregation. Drugs from an FDA approved drug library of 800 compounds were added at 10 ?M concentration and Sarkosyl-insoluble tau levels were analyzed. Results: So far, 52 compounds have been observed to significantly decrease tau aggregation with low cytotoxicity. These compounds include NMDA receptor antagonists, microtubule stabilizing taxane drugs, dihydropyridine type Ca^{2+} channel blockers, μ and κ opioid receptors modulators. Some Aβ aggregation inhibiting compounds were also observed to lower tau aggregation in this model as well. Additionally, these compounds were tested by using another case of AD seed, and different tauopathy (Corticobasal Degeneration [CBD] and Progressive supranuclear palsy [PSP]) seeds. Conclusion: We found drugs with different target pathways as AD tau aggregation inhibitor candidates. Moreover, these drugs varied in the effectiveness against different non-AD tauopathy seeds. These candidates will be tested in vivo and molecular mechanism of inhibition will be studied in future.

APe-01-2 Different peripheral immune modulation between Pink1$^{-/-}$ and Parkin$^{-/-}$ mice during EAE

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Objective: although the pathogenesis of neurodegenerative diseases is still widely unclear, mitochondrial dysfunctions and inflammation are thought to have a key role. Recent evidence has shown that PINK1 and PARKIN, two enzymes involved in mitophagy, play also a pivotal role in adaptive immunity. Methods: to elucidate their functions during neuroinflammation, Pink1$^{-/-}$ and Parkin$^{-/-}$ mice of different age groups were immunized with myelin oligodendrocyte glycoprotein peptide (MOG) $_{35\text{-}55}$ to develop experimental autoimmune encephalomyelitis (EAE). Results: compared to young wild type controls, Pink1$^{-/-}$ and Parkin$^{-/-}$ mice showed earlier disease onset. Parkin$^{-/-}$ mice displayed more severe acute symptoms, while Pink1$^{-/-}$ milder clinical score. Both middle-aged Pink1$^{-/-}$ and Parkin$^{-/-}$ mice showed an early onset and more severe acute phase than controls, with no EAE recovery in Parkin$^{-/-}$ mice. Aged (more than 1 year old) Parkin$^{-/-}$ mice developed an earlier onset and most severe EAE compared to the other group. In addition, aged Pink1$^{-/-}$ and Parkin$^{-/-}$ mice showed persistent disease during the recovery phase. These different clinical courses of EAE in these genetically modified mice were associated with variation in the percentage of IAIE^{+}CD11c^{+} dendritic cells and CD11b^{+}LY6G^{-} myeloid cells in the spleen. Conclusions: PINK1 and PARKIN proteins play an age-related role in the modulating of peripheral inflammatory response during EAE. The mechanisms involved aging, neuroinflammation and neurodegeneration, will open many potential avenues for the development of new therapies.

APe-01-3 withdrawn

APe-01-4 Driver gene KRAS mutation contributes to cancer-associated stroke aggravation via repressing MEK/ERK

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Purpose: Cancer-associated thrombosis (CAT) is a fatal clinical problem, and has attracted increasing public attention in the past decade. Currently almost 10% of patients with ischemic stroke have comorbid cancer, and this clinical frequency is expected to increase. Despite countless efforts in clinical prevention and treatment, the mechanism between oncogene mutation and ischemic stroke has not been fully elucidated. In this study, we focused on KRAS, a common mutated oncogene in various progressive cancers, and discuss the mechanism by which the mutant KRAS aggravated ischemic stroke. Methods: To build an experimental CAT model, we inoculated *KRAS* (G13D/-) HCT116 cells and parental HCT116 cells to C57BL/6 mice respectively, then subjected them to 20 min photochemically-induced thrombosis (PIT). Rotarod and Grid test were used to evaluate mice long-term neurological deficits. Western blotting assay and Enzyme-Linked Immunosorbent Assay (ELISA) were used to detect proinflammatory cytokines and neuroprotective proteins. Results: KRAS (G13D/-) HCT116 inoculated mice showed worsened function recovery after PIT in comparison with parental KRAS HCT116 inoculated mice. Additionally, KRAS (G13D/-) HCT116 inoculated mice showed enhanced proinflammatory cytokines, and suppressed MEK/ERK signaling pathway activation. Conclusion: KRAS (G13D/-) mutation aggravated mice stroke outcomes by promoting brain inflammation and repressing endogenous neuroprotective MEK/ERK signaling pathway. This study may provide a novel approach for cancer-associated stroke clinical treatment.

APe-01-5 An Alteration of microRNAs and Cognitive Impairment in Exercised Mice

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Objectives: Currently, emerging research interest is on the ability of microRNAs (miRNAs) to modulate the central nervous system function and pathophysiology of Alzheimer's Disease. Exercise can prevent and improve the pathophysiology of diseases and promote healthy aging. In the present study, we sought to investigate the alteration of miRNAs in voluntarily exercised mice. Methods: Ten C57BL/6 mice were separated to a ""wheel runner"" group with 5 months of exercise, and a ""sedentary"" group kept in the cage. Reference learning (acquisition) and memory (retention) were assessed by Morris water maze test and mice were sacrificed by cervical dislocation. The unilateral hippocampus was used for miRNAs and cytokine array analysis. Results: Behavior studies exhibited improvement of learning and memory after exercise. miRNAs microarray data analyzed by transcriptome analysis revealed 9 miRNAs (miR-695, miR-714, miR-190, miR-218, miR- 195, miR-7014, miR-6391, miR-6909, miR-6982) were upregulated and 8 miRNAs were downregulated (miR-434, miR-532, miR-467, miR-431, miR-669, miR-7075, miR-9, miR-764) upon exercise. Cytokine microarray data showed the number of inflammatory mediators, such as IL-1α, CRP, IFN-γ, TNF-α were decreased after the exercise. Conclusion: Taken together, we speculated miRNAs could be regulated in the inflammatory related to cognitive impairment. The further functional genomic study will be needed to detect signaling molecules responsible for the regulation miRNAs and cytokines.

APe-01-6 Deviation of BP from autoregulation limits after intravascular treatment correlates with outcomes

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Background Blood pressure (BP) management at the early time after endovascular thrombectomy (EVT) lacks evidence. Determination of cerebral autoregulation (CA) limits might guide BP management. Our study aimed to evaluate the association between outcomes and the duration as well as the degree of deviation of BP out of CA limits. Methods We enrolled patients who received EVT for acute anterior circulation stroke in our Hospital. Lower and upper limits of CA were determined by the BP at which the mean velocity index (Mx) was above the CA-impairment threshold. We calculated percent time and time-BP area that BP was out of the CA limits of each subject. The primary outcome was functional independence (mRS scored 0-2) at 90 days. The secondary outcomes were early neurological recovery (ENR, NIHSS scores reduced >4 at 7 days), infarct volume growth at 24 to 48 hours and symptomatic hemorrhagic transformation (sHT) within 7 days. Results A total of 102 patients were enrolled, and 94 were analyzed. Percent time and time-BP area with BP out of the CA limits were associated with functional independence at 90 days (aOR per 10% time 0.81 P=0.008, aOR per 10 h·mmHg 0.96 P=0.003). Time-BP area correlated with infarct volume growth (aOR per 10 h·mmHg 1.02 P=0.031). No correlation was noticed between percent time, time-BP area and ENR, sHT. Conclusion Infarct volume growth, functional outcome after EVT correlate with both duration and the extent of BP deviation from CA limits. Approaching the CA-preserved BP range might improve prognosis through maintaining proper cerebral perfusion.