AO-01-1 FUS suppresses RAN translation and neurodegeneration as an RNA chaperone in C9orf72-linked ALS/FTD

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[Objective] Abnormal expansion of GGGGCC (G_4C_2) repeat sequence in the noncoding region of the *C9orf72* gene is the most common cause of familial amyotrophic lateral sclerosis and frontotemporal dementia (C9-ALS/FTD). The transcribed G_4C_2 repeat RNA accumulates as RNA foci, sequestering various RNA-binding proteins (RBPs). Moreover, the G_4C_2 repeat RNA is also translated into dipeptide repeat proteins (DPRs) by noncanonical repeat-associated non-AUG (RAN) translation, which play central roles in the pathogenesis of C9-ALS/FTD. However, the mechanisms regulating RAN translation remain to be elucidated. **[Methods/Results]** We established C9-ALS/FTD *Drosophila* models expressing the expanded G_4C_2 repeat sequences, which showed degenerative phenotype and pathological features including DPR aggregation. We next performed the genetic screening for RBPs targeting G_4C_2 repeat RNA, and found that the ALS/FTD-linked RBP FUS suppresses RAN translation, resulting in the suppression of G_4C_2 repeat-induced degeneration *in vivo*. These suppressive effects were abolished by mutations in the RNA-recognition motif of FUS. Moreover, FUS was found to directly bind to G_4C_2 repeat RNA and modify its G-quadruplex structure as an RNA chaperone, resulting in the suppression of RAN translation *in vitro*. In addition, other G-quadruplex-targeting RBPs also suppressed G_4C_2 repeat-induced toxicity in our C9-ALS/FTD flies. **[Conclusions]** We revealed a novel regulatory mechanism of RAN translation by G-quadruplex-targeting RBPs, providing therapeutic insights for C9-ALS/FTD and other repeat expansion diseases.

AO-01-2 Progressive MS patients-derived gut bacteria induce neuronal inflammation via flagella-Th17 axis

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[Objectives] To identify the specific gut bacterial species associated with multiple sclerosis (MS) progression from RRMS to SPMS and reveal the mechanism which exacerbates neuronal inflammation. **[Methods/Results]** We analyzed the composition of fecal microbiomes between 62 RRMS patients and 15 SPMS patients based on the metagenomic sequencing data. We identified bacteria X whose abundance was significantly enriched in SPMS and positively correlated with a neurological disability score. Then we isolated bacteria X from the fecal samples of SPMS and RRMS patients and conducted the in vivo analysis to verify the functional significance of them. Mono-colonization of germ-free mice with SPMS-derived bacteria X (n=13) caused severe neurological disability (p=0.0007) after immunization with significantly increased T helper 17 (Th17) cells in the central nervous system and large intestine lamina propria compared with germ free mice (n=13) or RRMS-derived bacteria X mono-colonized mice (n=13). In the genome comparison among various bacteria X strains, the genome of SPMS-derived bacteria X specifically included flagellar genes. The presence of flagella in SPMS-derived bacteria X was confirmed by electron microscopic images and in vitro co-culture experiments. A specific type of flagella is reported to be a selective agonist of toll-like receptor 5 and potentially induces Th17 cells via the secretion of interleukin-6 from the intestinal dendric cells. **[Conclusions]** Bacteria X enriched in the gut of SPMS patients might exacerbate neuronal inflammation via flagella-Th17 axis.

AO-01-3 A centrally acting connexin hemichannel blocker attenuates multiple system atrophy-cerebellar type

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Objective: Multiple system atrophy (MSA) is an intractable neurodegenerative disease characterized by accumulation of phosphorylated α-synuclein (p-α-syn) in oligodendroglia. Connexin (Cx) gap junctions allow intercellular communication between adjacent glia to maintain homeostasis in the central nervous system while Cx hemichannels (HCs) release ATP and pro-inflammatory cytokines that facilitate neuroinflammation. We aimed to elucidate therapeutic effects of INI-0602, a centrally acting Pan-Cx HCs blocker, in a novel MSA-C model. **Methods:** We generated TetO-α Syn A53T Tg/+; PLP-tTA Tg/+ double transgenic (Tg) mice expressing mutant human A53T α Syn in oligodendroglia when doxycycline was removed from the diet. We pathologically studied Cx expression patterns in Tg mice at 16, 24, and 30 weeks of age. Acute slices were evaluated for ethidium bromide (EtBr) uptake. We intraperitoneally injected INI-0602 (20 mg/kg) or vehicle in Tg mice (n=8 in each group) every other day from 18 weeks of age and evaluated body weight, clinical score, and rotarod performance. **Results:** Tg mice showed rapidly progressive ataxia from 22 weeks of age and had p-α-syn accumulation, demyelination and neuronal loss with activated microgliosis and reactive astrogliosis in the brainstem/cerebellum. Astrocytic Cx43/Cx30 and oligodendrocytic Cx47/Cx32 were extensively diminished along with p-α-syn accumulation. INI-0602 inhibited EtBr uptake. INI-0602 treatment improved clinical scores and rotarod performance compared to vehicle treatment. **Conclusion:** INI-0602 attenuates MSA-C progression in Tg mice.

AO-01-4 Long-read sequencing reveals allelic heterogeneity of repeat expansion in OPDM

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[Objective]The pathogenesis of non-coding repeat expansion disorders is not fully understood, although toxicities of the expanded RNAs or repeat-associated non-AUG (RAN) translated proteins have been suggested. To elucidate the pathomechanism in oculopharyngodistal myopathy (OPDM), we aimed to know the precise sequences of CGG repeats within the expanded alleles in 5'UTR of *LRP12*, *GIPC1*, *NOTCH2NLC* and *RILPL1*, using long-read sequencing (LRS) at a single molecular level. **[Methods]** CRISPR/Cas9-targeted LRS were performed on Nanopore MinION™ sequencer by cutting with designed guideRNAs to isolate CGG expanded regions in the above 4 genes. Basecalling and sequence alignment were carried on Guppy and Minimap2. Genetically confirmed 58 OPDM patients (*LRP12*: n= 49, *GIPC1*: n= 4, *NOTCH2NLC*: n=5) were analyzed. **[Results]**We successfully got a certain number of reads regardless of expansion (17 ± 14 /sample) with 100% of sensitivity and specificity. The number of expanded reads in affected genes was about half of the total reads (47 ± 13%), indicating heterozygosity in OPDM. Interestingly, even within the individual patient, the repeat length differed from allele to allele. Also, the expanded reads frequently had insertions and deletions, indicating that the reading frame in RAN-translated product would not be maintained. **[Conclusion]**We have established a diagnostic method for OPDM with high accuracy. The heterogeneity in the size and composition of expanded alleles suggests pathomechanism of OPDM is not so simple as toxicities of the homogeneous expanded RNAs or RAN translated products.

AO-01-5 Development of novel neuropathic pain treatments targeting SEMA-Plexin pathway

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[Objective] Semaphorins (SEMAs) and their receptors, Plexins, are expressed in pain-conducting neurons of dorsal root ganglia (DRG). However, it remains unclear their association with neuropathic pain (NP). Herein, we aimed to clarify the association between NP and SEMA-Plexin pathway. **[Methods]** We quantified serum SEMA3A, 3E, 4A, 4D, and 7A in 45 patients with NP and 17 age- and sex-matched healthy controls (HCs) by enzyme-linked immunosorbent assay (ELISA). SEMA expression in DRG and peripheral nerve (PN) tissues of 4 autopsied NP patients and 2 controls as well as NP model mice with partial sciatic nerve ligation (PSL) was assessed by immunohistochemistry (IHC). Moreover, we intraperitoneally injected anti-SEMA blocking antibody or control IgG into NP model mice for 5 consecutive days after PSL, and assessed mechanical hypersensitivity using von Frey filament on day 4 after PSL. **[Results]** Serum ELISA showed a significant increase of the SEMA3E, a ligand of Plexin D1, in NP patients compared to HCs. IHC revealed enhanced SEMA3E expression in DRG and PN tissues of both NP patients and NP model mice, especially in macrophages. Injection of anti-SEMA3E blocking antibody not only abolished activation of DRG macrophages but also resolved hypersensitivity in NP model mice. In vitro, treatment with SEMA3E inhibited neurite outgrowth of mouse dissociated DRG neurons, whereas co-treatment with SEMA3E and anti-SEMA3E blocking antibody induced neurite outgrowth. **[Conclusions]** SEMA3E/Plexin D1 pathway was associated with NP and the blockade of SEMA3E could be a novel NP treatment.

AO-01-6 Tim3 critically regulates microglial function and accelerates Alzheimer's disease pathology

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[Objective] Microglia (MG) get activated (MGnD phenotype) in Alzheimer's disease (AD) and phagocytose / clear neurotoxic amyloid beta (Aβ). *Havcr2*, encoding a checkpoint molecule, Tim3, was recently found as a susceptibility gene in AD. We aim to find its as-yet-unknown role in AD. **[Methods]** MG-specific Tim3-deficient ($Havcr2^{cKO}$) mice were crossed to an AD model, 5XFAD (n=75). Behavior test, immunohistology, and snRNAseq were performed. Intracellular mechanism was further explored with molecular analyses including IP-MS screening. **[Results]** Tim3 expression was strikingly high in MG. Tim3-deficient MG showed enhanced phagocytotic ability *in vitro* and *in vivo*, with a transcriptome reminiscent of MGnD and TGF-β signaling-deficient MG. Consistently, $Havcr2^{cKO}$ 5XFAD mice showed no cognitive impairment, with less Aβ plaque load (52.5% reduction) and less neuronal injury (29.0% reduction), where snRNAseq identified a unique hyper-phagocytotic MG subset. IP-MS screening and confirmatory IP-WB showed that Tim3 binds Smad2, a key molecule in TGF-β signaling. Tim3 enhanced phosphorylation of Smad2, which required their binding using the C terminus of Tim3. Besides, motif enrichment analysis identified Smad2 as a core transcription factor regulating Tim3-deficient MG transcriptome. **[Conclusion]** Tim3 is a key regulatory molecule that inhibits proper activation and phagocytotic function of MG by enhancing TGF-β signaling, thereby inhibiting clearance of Aβ plaques in a preclinical model of AD. Tim3 may be a promising target of a new therapeutic strategy for this intractable disease.

AO-02-1 Serum/urine exosomal microRNA-203 is a novel least-invasive biomarker for early Alzheimer's disease

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Objective: As Alzheimer's disease (AD) is rapidly increasing, convenient and reliable biomarkers to diagnose AD in the early stage become more critical than ever. We found increased microRNA (miR)-203 and decreased miR-30c-2 levels in serum exosomes in APP-KI AD model mice upon disease onset. Based on these findings, we aimed to develop blood and urine exosomal miR biomarkers for AD diagnosis and disease progression. **Methods:** Blood and urine samples were collected from 15 AD patients and 15 age- and sex-matched healthy controls (HCs). Exosomal RNA was extracted from serum and urine using ExoQuick® Exosome Isolation and RNA Purification kits, and an exoRNeasy® Midi kit, respectively. Each candidate miR was validated by qPCR assays using TaqMan microRNA assays. Each miR amount was normalized to cel-miR-39. Relative expression levels of miR were calculated by a 2-ΔΔCt method. **Results:** Serum exosomal miR-203 levels significantly increased and miR-30c-2 levels decreased in AD patients compared with HCs. Thus, the miR-203/30c-2 ratio provided 86% sensitivity and 100% specificity for AD diagnosis, and negatively correlated with Mini-Mental State Examination scores ($p < 0.001$). In urinary exosomes, miR-203 increased in AD patients compared with HCs ($p < 0.05$) but miR-30c-2 was not detected in any cases. **Conclusion:** We consider that the plasma exosomal miR-203/30c-2 ratio is a novel blood biomarker specific for early diagnosis and disease progression of AD. We also propose that urinary exosomal miR-203 could be a potential non-invasive AD biomarker, which can be repeatedly examined.

AO-02-2 Unedited GluA2 mRNA in cerebrospinal fluid: A promising biomarker for sporadic ALS

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[Background] With an increase in clinical trials, the development of an effective ALS therapy is realistic in near future; then, exploitation of reliable biomarkers is increasingly needed. Reduced RNA editing of GluA2 mRNA due to down-regulation of adenosine deaminase acting on RNA 2 (ADAR2) in the lower motor neurons has been demonstrated as a disease-causing molecular abnormality in sporadic ALS patients. As the editing efficiency at ADAR2-dependent sites in extracellular RNAs reflects cellular ADAR2 activity, we investigated the changes in the editing efficiency of CSF RNA. [Materials and Methods] We measured the editing efficiency at the glutamine/arginine (Q/R) site of GluA2 mRNA in 1 ml of frozen-stocked CSF from 14 sporadic ALS patients and 16 non-ALS patients as controls, and subsequently investigated the correlation between editing efficiency and clinical parameters. [Results] Editing efficiency was significantly reduced in sporadic ALS patients compared to controls (P=0.015, Mann-Whitney U test). The editing efficiency was greater than 95% in all controls, and a threshold of 94.6% yielded a sensitivity of 100% for discrimination between ALS and non-ALS patients. Further, the editing efficiency significantly correlated with ALSFRS-R (P=0.045), especially in the gross motor functions subgroup (P=0.003, Spearman correlation coefficient). [Conclusions] Detection of Q/R site-unedited GluA2 mRNA in the CSF is a potential disease-specific diagnostic biomarker for sporadic ALS, and may also serve as a biomarker to predict disease progression and treatment efficacy.

AO-02-3 Novel glycobiomarkers defining therapeutic response in CIDP

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【目的】慢性炎症性脱髄性多発神経炎（CIDP）の発症機序や病態は依然として不明であり、十分な病態解明には至っていない。そこで、我々は髄鞘形成や神経炎症に関わる糖鎖に注目し、CIDP患者血清の網羅的糖鎖解析を行うことで、病態ならびに治療反応性を規定する新規糖鎖バイオマーカーの探索を行った。【方法】対象は2012年から2021年までにEAN/PNS診断基準により当院で診断したCIDP連続81例のうち、初診時に未治療であった典型的CIDPを対象とした。グライコプロッティング法およびビラゾロン共存下β脱離反応により、治療前血清の*N*結合型糖鎖、*O*結合型糖鎖について網羅的に解析した。健常者血清を対照群として、CIDP患者血清における糖鎖の変化を捉え、各臨床所見との関連性を検討した。【結果】健常対照群20例（男性11例、年齢中央値66歳）および典型的CIDP群27例（男性14例、年齢中央値56歳、平均罹病期間6ヶ月）の血清を解析した。対照群と比較しCIDP群で、*N*結合型糖鎖およびシアル酸含有酸性複合型糖鎖の総量で有意な低下を認めた（p<0.05）。個別の糖鎖では、α2,3結合型シアル酸含有糖鎖の値を用いることではAUC 0.848でCIDPの識別が可能であった。*O*結合型糖鎖では、総量、シアル酸の結合様式の群別で比較し、対照群との間に有意差は認めなかった。治療反応性に関しては、治療前の*N*結合型糖鎖、酸性複合型糖鎖およびα2,6結合型シアル酸含有糖鎖の低値が初回IVIgへの治療抵抗性と有意な関連を認めた（p<0.05, p<0.01, p<0.05）。【結論】典型的CIDPでは*N*結合型糖鎖および酸性複合型糖鎖量は健常者と比較し、有意に低下しており、初回治療への反応性を予測する新規のバイオマーカーとなる可能性がある。

AO-02-4 Intracerebral hemorrhage severity and outcome taking antithrombotic agents: Japan Stroke Data Bank

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【目的】直接作用型経口抗凝固薬（DOAC）はワルファリン（WF）に比して抑制する凝固因子が少なく半減期が短い．DOAC内服中に発症した脳内出血例ではWF内服中に発症した例に比して発症時重症度が低く転帰良好である可能性がある．脳内出血例における発症時抗血栓薬と発症時重症度および転帰との関連についての検討した．【方法】日本脳卒中データバンク参加施設に2017年1月から2020年12月に発症24時間以内の脳内出血で入院した例を対象とした．発症時内服していた抗血栓薬により，非内服群，抗血小板薬群，WF群，DOAC群の4群に分類した．非内服と比して各抗血栓薬が入院時National Institutes of Health Stroke Scale（NIHSS）と退院時転帰不良（modified Rankin Scale（mRS）5-6）に関連するか評価した．【結果】脳内出血9,948例（女性：4,329例，年齢70±15歳）のうち，非内服は7,698例，抗血小板薬は1,290例，WFは389例，DOACは571例であった．入院時NIHSSは各群で中央値12（四分位範囲：5-22），13（5-26），15（5-30），13（6-24）であった．多変量解析では，非内服に比してWFは入院時NIHSS高値に関連していたが（incident rate ratio :1.08[95％信頼区間 1.05-1.12]），抗血小板薬，DOAC内服は有意な関連を認めなかった（抗血小板薬1.01[0.99-1.03]，DOAC0.97[0.94-1.00]）．退院時mRS5-6の症例の割合は非内服2,369例（30.8％），抗血小板541例（41.9％），WF189例（48.6％），DOAC237例（41.5％）であった．多変量解析では，非内服に比してWF内服は退院時mRS5-6に有意に関連していたが（オッズ比:1.79[95％信頼区間1.23-2.60]），抗血小板薬，DOACは有意な関連を認めなかった（抗血小板薬1.11[0.90-1.36]，DOAC1.27[0.90-1.78]）．【結論】脳内出血発症時に抗血栓薬を内服していない症例と比較して，WF内服中に発症した症例は入院時重症で，退院時転帰不良が有意に高率であった．DOAC内服は抗血栓薬なしの症例と重症度や転帰に有意な差を認めなかった．

AO-02-5 NCVC GENOME Registry: Rationale and baseline characteristics for RNF213 variant carriers

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[Objective] We aimed to clarify the association between the *ring finger protein 213* (*RNF213*) p.R4810K variant and each ischemic stroke (IS) subtype.**[Methods]** The NCVC Genome Registry is a prospective multicenter observational study enrolling patients with a history of IS, hemorrhagic stroke, transient ischemic attack, asymptomatic stroke, or stroke mimics, and those with informed consent for the *RNF213* genotyping testing between 2015 and 2022. We extracted the patients with previous IS, divided them into two groups (variant carriers and non-carriers), and compared the patient characteristics between both groups. The IS subtypes were classified into atherosclerosis, small-vessel occlusion, cardiac pathology, other causes, and dissection according to ASCOD classification.**[Results]** Of 2501 patients enrolled, 1815 IS patients (642 females, 73 years of median age) were analyzed. Compared to the non-carriers (n=1759), the variant carriers (n=56) were younger (63 years vs. 74 years, P<0.01), less frequently had hypertension (67.9% vs. 80.2%, P=0.03), more frequently M1 segment of middle cerebral artery steno-occlusion (39.3% vs. 13.2%, P<0.01), and more frequently had atherosclerosis (42.9% vs. 19.8%, P<0.01), while the other IS subtypes did not differ significantly between both groups. In multivariable logistic regression analysis, atherosclerosis was associated with the variant carriers (adjusted odds ratio 1.72, 95% confidence interval 1.04-2.84).**[Conclusions]** The *RNF213* p.R4810K variant carriers were significantly more prevalent in atherosclerosis than in the other IS subtypes.

AO-02-6 Diagnostic implications of MOG-IgG detection in sera and cerebrospinal fluids

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[Objective] Spectrum of myelin oligodendrocyte glycoprotein (MOG) IgG-associated disease (MOGAD) includes optic neuritis (ON), myelitis (MY), acute disseminated encephalomyelitis (ADEM), cerebral cortical encephalitis (CE), and aquaporin-4-IgG (AQP4-IgG)-negative neuromyelitis optica spectrum disorder (NMOSD). MOG-IgG are usually detected in sera, but the diagnostic implications of the MOG-IgG in sera and CSF remains unclear. [Methods] In this cross-sectional study, we identified patients with paired serum and CSF sent from all over Japan for testing MOG-IgG. Two investigators blinded to MOG-IgG status classified them into suspected MOGAD or not based on the current recommendations. We analysed the relations between MOG-IgG in sera and CSF, and the phenotypes with multivariable regression. [Results] A total of 671 patients before treatment were tested. In 405 suspected MOGAD, 133 patients (33%) tested MOG-IgG-positive in serum and/or CSF; 94 (23%) double-positive, 17 (4.2%) seropositive and CSF-negative, and 22 (5.4%) CSF-positive and seronegative. The specificity of MOG-IgG in sera and MOG-IgG in CSF in suspected MOGAD were 100% (95%CI 99%-100%) and 99% (97%-100%). Multivariable regression analyses revealed MOG-IgG in CSF were independently associated with ADEM and CE. [Conclusions] Our study showed that 70% of MOGAD cases were MOG-IgG-positive in both sera and CSF, but in the remaining cases, MOG-IgG was detected in either sera or CSF alone. Different MOG-IgG detection patterns in blood and CSF appear to be linked with MOGAD clinical phenotypes.

AP-01-1 A new strategy for DMD exon skipping with RNA-DNA hetero-G4 structure inducing ASOs

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Objective: Duchenne muscular dystrophy (DMD) is a fatal X-linked disorder caused by nonsense or frameshift mutations in the DMD gene, resulting in a loss of normal dystrophin production. Therapies by antisense oligonucleotides (ASOs)-mediated exon skipping and correcting a shift of the amino acid are the leading approach for restoring dystrophin expression. However, these therapies still have only limited effects in DMD patients. Our aim is to develop novel strategies for exon skipping by modulating the secondary RNA structures with ASO. Herein, we report newly designed guanine-rich ASO (G-ASOs) for exon skipping, which introduce the formation of an RNA-DNA hetero-G-quadruplex (G4) structure in pre-mRNA. These G4 structures inhibit RNA splicing by spliceosome and enhance exon skipping *in vitro* and *in vivo*. Methods: To determine whether G-ASO induce G4 in RNA template comprising G tracts, *in vitro* stop assay for *RTase* activity and ThT binding assay for G4 structures were performed. In addition, we have newly developed a EGFP-based reporter assay system to detect fluorescence only when skipping of mdx-type exon 23 is induced by ASOs. Finally, we evaluated the effects of exon skipping in the mdx mice treated with G-ASO by intramuscular injection. Results: We confirmed the exon 23 skipping by the formation of G4 and the restoration of dystrophin expression level with the administration of the G-ASOs. Conclusion: Our data highlight the potential application for exon skipping with G-ASOs and allow us to propose a novel therapeutic approach for neurological diseases.

AP-01-2 Characterization of CHCHD2 variants linked to amyotrophic lateral sclerosis and Parkinson's disease

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[Objectives]The purpose of this study was to analyze the roles of CHCHD2 pathogenic variants. [Methods]We performed the genetic analysis for CHCHD2 among 944 ALS patients and 1091 PD patients. Histochemical analysis and WB were performed using the brain tissues with CHCHD2 variants. We generated stably expressing CHCHD2 WT, P14L and T61I in *CHCHD2* deficient SH-SY5Y cells. *Drosophila* models expressing CHCHD2 and its pathogenic variants which were also generated. [Results]We newly identified CHCHD2 -8T>G and P14L variants from ALS patients and P14L variant from PD patients. We observed the reduction of CHCHD2 and CHCHD10 in the brain of ALS patient with P14L. Immunostaining and WB analysis of SH-SY5Y cells revealed the dissociation of P14L from the mitochondria to the cytoplasm. The similar results of P14L were observed in *Drosophila* neurons. Immunostaining and WB analysis of *CHCHD2*$^{-/-}$ SH-SY5Y cells showed higher phospho-TDP-43 aggregation. The similar results were obtained in *Drosophila* neurons. CHCHD2 P14L showed the reduction in mitochondria Ca^{2+} uptake and the increase in cytoplasm Ca^{2+}flow in *Drosophila* neuron terminals. The similar results were obtained in SH-SY5Y cells. [Conclusions]We identified CHCHD2 variants from both ALS and PD cohorts. The early mitochondrial dissociation of CHCHD2 P14L suggests to affect the dysregulation of Ca^{2+} uptake into mitochondria, leading to Ca^{2+} overflow into the cytoplasm. The dysregulation of Ca^{2+} handling is likely to cause the excitotoxicity of motor neuron and dopamine neuron, leading to TDP-43 proteinopathy and synucleinopathy.

AP-01-3 Iguratimod blocks glial IL-6 production and Th17 migration in a progressive multiple sclerosis model

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Objective: We reported a novel secondary progressive multiple sclerosis (SPMS) model; experimental autoimmune encephalomyelitis (EAE) in oligodendroglia-specific *Cx47*-inducible conditional knockout (*Cx47* icKO) mice. As we reported the efficacy of iguratimod (IGU), an anti-rheumatic drug, on acute EAE, we aimed to elucidate the effects of IGU on the SPMS model. **Methods**: *Cx47* icKO mice were immunized with MOG_{35-55} to induce EAE. IGU or vehicle was administered from 17 to 50 days postimmunization. We prepared in vitro mixed glial cell culture, and measured cytokine levels in the culture supernatant after stimulation (IL-1a, C1q, TNF-a, and LPS) ± IGU. We performed $CD4^+$T cell migration assay toward the culture. **Results**: EAE scores and demyelinated areas were markedly attenuated in IGU-treated mice in chronic phase ($p<0.0001$ and $p=0.011$, respectively). Areas of $CD3^+$ T cells, $F4/80^+$ macrophages, $NOS2^+Iba1^+$ microglia, and $C3^+GFAP^+$ astroglia in the lumbar spinal cord lesions were less in IGU-treated mice than vehicle-treated mice ($p<0.05$ for all). CSF IL-6 levels were decreased by IGU treatment ($p<0.01$). Flow cytometric analyses of the CNS tissues revealed that migrated Th17 cells were decreased by IGU treatment ($p<0.05$). The migration assay disclosed reduction of Th17 migration by IGU treatment ($p<0.05$). IL-6 and CCL2 levels in the culture supernatant were decreased in IGU-treated group compared to vehicle-treated group ($p<0.05$ and $p<0.01$, respectively). **Conclusion**: IGU mitigates SPMS model severity by suppressing Th17 migration through inhibiting IL-6 production from glial cells.

AP-01-4 Vitamin B12 may inhibit the neurotoxicity of Amyloid beta oligomers and protect memory function

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[Objective] Amyloid beta oligomers (Aβo) play the primary role in Alzheimer's disease (AD). Recently, we have noted that vitamin B12 (VB12) may preserve cerebral blood flow in memory-related regions in patients with cognitive impairment. In this study, we investigated the effects of VB12 on cognitive function and cortical structures. Further, we examined the mechanism of the protective effect of VB12 against Aβo neurotoxicity, evaluating both basic and clinical aspects.**[Methods]** SPECT, high-resolution MRI, and serum VB12 levels were measured in 153 patients (50-98 years old) who visited our memory clinic from Apr. 2016 to Sep. 2020. Cortical structures were analyzed by measuring thickness and fractal dimension (FD) using surface-based morphometry. The protective effect of VB12 against neuronal cell injury induced by 5 μM Aβo was evaluated using human neuroblastoma cells (SH-SY5Y cells).**[Results]** We found an association between VB12 and hippocampal cerebral blood flow and an association between VB12 and cortical thickness and FD in the medial temporal lobe. In cellular experiments, Aβo decreased MnSOD and mitochondrial membrane potential difference, increased ROS, plasma membrane phospholipid peroxidation capacity, oxidized glutathione generation, and decreased cell viability; VB12 decreased these oxidative stresses and significantly inhibited Aβo-induced cell injury.**[Conclusion]** VB12 suppressed Aβo-induced cell injury by its strong antioxidant effect, suggesting that VB12 may be helpful for the protection of brain microstructures in AD.

AP-01-5 Human brain organoids and fetal brain contain similar neuronal populations at single-cell resolution

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[Objective] Single-cell RNA-sequencing (scRNA-seq) technologies allow us to identify homologous cell types and the molecular heterogeneity between individual cells. Previously, we established human brain organoids of brainstem (hBSOs) and midbrain (hMBOs), and performed scRNA-seq on these organoids. The objective of the present study is to compare these organoids and human fetal brain at single-cell resolution. [Methods] The scRNA-seq datasets of hBSOs and hMBOs were integrated and analyzed. The comparative analysis was performed between these organoids and publicly available scRNA-seq dataset of human fetal central nervous tissues. [Results] The scRNA-seq datasets of the two organoids (2,345 cells at hBSOs and 1,295 cells at hMBOs) and publicly available scRNA-seq dataset of human fetal midbrain (hfMB, 1977 cells) were integrated and clustered into 12 cell populations. The two organoids contained neuronal populations (164/2,345 cells at hMBOs and 845/1,295 cells at hMBOs) as that of hfMB (626/1,977 cells at hfMB). Euclidean distance matrix between brain organoids and human fetal central nervous tissues shows that the distance of hfMB to hMBO shows shorter than that of hfMB to hBSO (7.83 and 16.06 respectively). [Conclusions] The scRNA-seq analysis showed that the hBSOs and hMBOs contained similar neuronal populations as that of hfMB. Human brain organoids are expected to be useful tools for disease modeling to elucidate the pathology of neurodegenerative disease.

AP-01-6 Helweg's triangular tract degeneration in multiple system atrophy

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[Objective] Multiple system atrophy (MSA) is an adult-onset neurodegenerative disorder characterized by alpha-synuclein pathological aggregation in glia and neurons. Helweg's triangular tract (HT) was described in detail in 1888. This mysterious tract exists in the human medulla oblongata and at least the first five cervical segments. It is unknown if certain neurological disorders cause HT degeneration. Here, we studied HT degeneration in neuropathologically established MSA. [Methods] The clinicopathological findings of 201 autopsied patients with MSA were reviewed. 104 patients were excluded due to insufficient clinical or pathological data. The remaining 97 patients' upper cervical cord was evaluated. HT degeneration was defined as myelin sheath loss with a triangle-shaped configuration within the anterior and lateral funiculi boundary, bilaterally, which continuously appeared from C1 to C4. The spinal cord of 20 controls and 30 patients with Lewy body disease, amyotrophic lateral sclerosis, and progressive supranuclear palsy were also assessed. [Results] Among the 97 patients with MSA, 22 patients (22.7%) showed HT degeneration in upper cervical cord. Controls and patients with other neurodegenerative disorders did not exhibit definitive HT degeneration. The 22 patients with HT degeneration showed a younger disease onset, longer disease duration, and more severe semiquantitative scale of olivopontocerebellar atrophy than the other 75 patients. [Conclusions] HT degeneration may be a characteristic neuropathological finding of the upper cervical cord in MSA.

AP-01-7 Chronic sleep fragmentation accelerates prodromal disease course in Parkinson's disease model mice

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[Objective] Sleep fragmentation due to frequent nocturnal arousal is prevalent among older adults and patients with Parkinson's disease (PD). Recent clinical studies suggested that chronic sleep fragmentation could exacerbate PD. However, their causal relationship remains unknown. We aimed to test whether sleep fragmentation exacerbates the symptoms and pathology of PD using a prodromal mouse model. [Methods] 10- to 11-weeks-old A53T *SNCA* bacterial artificial chromosome transgenic (Tg) or wild type (WT) mice were housed in either (1) sleep disturbance (SD) cage (SW-15SD; Melquest), a running-wheel-based device that induces chronic sleep fragmentation (SD group; n=31), (2) wheel cage (WC), a control cage with a running wheel but without sleep disruption (WC group; n=32), or (3) normal cage (NC), another control cage without running wheel (NC group; n=27). After 5, 6, or 7 weeks, constipation, hyposmia, or phosphorylated α-synuclein (p-syn) in the Tg mice brain sections were evaluated. [Results] Among the Tg mice, hyposmia and constipation were present only in the SD group. In WT mice of the SD group, no hyposmia was present; mild constipation was present but was significantly milder compared to the Tg mice in the SD group. In the Tg mice of the SD group, proteinase-K-resistant p-syn was increased in the hippocampus and piriform cortex. [Conclusion] Chronic sleep fragmentation accelerated the onset of hyposmia and constipation and p-syn accumulation in the prodromal PD model mice. Our results implicate that sleep fragmentation could serve as a new target to delay PD onset.

AP-02-2 Structural brain abnormalities common to visual hallucinations and abnormal BP fluctuations in LBD

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【目的】幻視（VH）を発症したレビー小体病（LBD）患者では、幻視未発症に比べ、血圧の異常変動が多いとの報告がある。LBDの心血管系調節障害には末梢交感神経だけでなく、中枢自律神経網の変性も関与することから、本研究では中枢自律神経網に注目し、同部位の変性が心血管自律神経障害のみならず幻視と関連するか、Voxel vased Morphometry（VBM）を用いて検討した。【方法】症例対照研究。対象はLBD患者102名（PD 97名、DLB 5名）。男性49%、年齢72.3±8.5（平均±SD）歳、罹病期間7.9±5.9年、ヤール重症度 3（中央値）。対象を幻視未発症（n VH）、minor VH発症（m VH）群、formed VH発症（f VH）群に分けた。心血管自律神経障害の指標として自由行動下収縮期血圧変動の標準偏差（SBP-SD）を収集し、3分割した。夜間血圧下降の程度も収集した。f VHと血圧変動との関連を多変量解析で検討した。中枢自律神経網とされる領域に関心領域（ROI）を置き、各ROIの萎縮の程度をf VH群とn VH群、SBP-SD高値群と低値群、riser statusの有無群で各々比較した。脳全体量の正規化には頭蓋内容積を用いた。【結果】n VH群、m VH群、f VH群はそれぞれ44、21、37名。年齢、性別、罹病期間で調整後、f VHはSBP-SD増大、riser statusと有意な関連があった（SBP-SD; 調整後OR=2.12（95%CI 1.4-3.2）, riser status; 2.79（1.2-6.7））。性別、年齢、罹病期間を共変量としたVBM解析では、SBP-SD高値群は低値群に比して、両側視床下部、扁桃体、視床枕、右角回、縁上回で、riser群は非riser群に比して、右視床下部、視床内背側核、海馬で有意な萎縮がみられた。上記共変量にMMSEスコアを加えた解析では、f VH群はn VH群に比して、右視床下部、左扁桃体に有意な萎縮がみられた（いずれもFWE補正 p<0.05, k=30）。【結論】LBDでは異常な血圧変動と幻視発症とには有意な関連がみられ、中枢自律神経網のうち視床下部と扁桃体の変性が両症候に共通している可能性がある。

AP-02-4 The association between serum IgG antiganglioside antibodies and poor outcome in GBS

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Objective:Approximately 20% of patients with Guillain-Barré syndrome (GBS) at 6 months from the onset remain to be unable to walk independently. Using modified Erasmus GBS outcome score (mEGOS), high score can predict such patients with poor outcome. We previously reported serum IgG-GD1a antibodies were associated with poor outcome and the combination of IgG-GD1a antibody positivity and high mEGOS could predict poor outcome more accurately than mEGOS alone. This study aimed to validate whether serum IgG-GD1a antibodies or GM1 antibodies were associated with poor outcome in another GBS cohort. Methods:We retrospectively collected 334 GBS patients with any IgG antiganglioside antibodies examined in our laboratory.Poor outcomes were defined as ≧ 3 of GBS disability score at 6 months. Results:Serum IgG-GM1 and GD1a antibodies were positive in 48% and 37% of 334 patients. We used multivariable logistic regression analysis to evaluate the relationships between IgG-GM1 or GD1a antibodies and poor outcomes. IgG-GD1a antibodies were independently associated with poor outcomes (OR 2.6, 95% CI:1.24 to 5.31, p=0.01). In contrast, IgG-GM1 antibodies were not associated with poor outcomes. IgG-GD1a-positive patients were more significantly associated with poor outcomes than IgG-GD1a-negative patients (19% vs 9%, p=0.015). Patients with the combination of IgG-GD1a antibody positivity and high mEGOS (either on admission or on day 7) had poor outcomes at the rate of 55% and 59%. Conclusion:The presence of IgG-GD1a antibody,but not IgG-GM1 antibody,is independently associated with poor outcome.

AP-02-1 Clinical presentation of cerebello-brainstem form of adrenoleukodystrophy and efficacy of HSCT

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【目的】副腎白質ジストロフィー（ALD）は*ABCD1*遺伝子変異によるX連鎖性遺伝性疾患で、中枢神経の白質や副腎が障害され、adrenomyeloneuropathy（AMN）、大脳型、小脳・脳幹型を含む多彩な臨床像を呈する。その中で、小脳・脳幹型ALDは脳幹や小脳の脱髄を来たし、経過2年で約半数が予後不良の大脳型ALDに移行する。大脳型では造血幹細胞移植(HSCT)が進行停止に有効で、小脳・脳幹型でも治療選択肢となるが、症例の蓄積は少ない。今回、小脳・脳幹型ALDの臨床的特徴やHSCTの治療効果について検討した。【方法】2006年～2022年の診断例で、小脳・脳幹型ALDの臨床像を呈したことのある症例の臨床経過、検査所見、治療効果について、単施設で後ろ向きに検討を行った。【結果】症例は7例で、平均発症年齢29歳(18-39歳)、平均経過観察期間4.8年(1.1-6.8年)であった。2例がAMN、5例が小脳・脳幹型として発症して、全例で小脳失調が進行した。小脳・脳幹型と診断した時点の頭部MRIで小脳7例、脳幹聴覚路3例、脳幹錐体路4例にT2高信号病変を認め、3例で造影増強効果を伴い、電気生理学的検査では全例でsubclinicalなABR異常を認めた。HSCT施行例は5例で、経過観察期間の中央値は4.5年（1.1-6.8年）だった。移植前後のEDSS, ALD-DRS, Barthel index, Loes scoreの中央値は、4.0→6.5, 2→2, 100→95, 5→5と一部で悪化したが軽度で、長期的には病勢は安定した。頭部MRIでは、4例で観察期間内の病変拡大はなく、1例で移植1年後に拡大したが、その後は縮小した。造影増強効果を伴った2例では、移植2か月後に所見の消失あるいは不明瞭化を確認できた。EDSS, ALD-DRS, Barthel index, Loes scoreを用いて、HSCT施行前の病勢の進行速度を大脳型16例と比較したところ、本病型での増悪はより緩徐であった。【結論】小脳・脳幹型ALDでは、subclinicalなABR異常を認め、大脳型よりも緩徐に進行した。また、HSCTの長期的な治療効果が示された。

AP-02-3 Reproducibility of serum alpha-synuclein seed detection method

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Objective: In the pathomechanisms of synucleinopathies such as Parkinson's disease (PD) and multiple system atrophy (MSA), the alpha-synuclein (aSyn) seed play a vital role in transforming a native form of aSyn to an aberrant conformational structure and attaining propagative characteristics. In the current investigation, real-time quaking-induced conversion (IP/RT-QuIC)-based immunoprecipitation was used to detect aSyn seeds in serum for the first time. Also, we analyzed the reproducibility of IP/RT-QuIC. **Methods**: Sera were collected from 35 participants with synucleinopathies (20 PD and 15 MSA), 20 controls as an external second cohort. In pathologically confirmed cases, sera were collected from 6 patients with synucleinopathies (3 PD and 3 MSA), 3 controls as an internal cohort, and one PD patient and two non-synucleinopathies as an external cohort. We performed IP/RT-QuIC and assay reproducibility was tested using the kapper coefficient. **Results**: IP/RT-QuIC had the best diagnostic performance for differentiating PD and MSA from controls. The intra-batch and inter-batch kappa coefficients of variation were 0.61-1.00. Positivity rates of IP/RT-QuIC for the cases with the pathologically confirmed diagnosis of the internal cohort-nonsynucleinopathy, MSA, and PD patients were 0%, 33%, and 100%, respectively. For the external cohort, non-synucleinopathy patients had 0% positivity rates, while PD patients had 100% positivity rates. **Conclusion**: This study demonstrated that IP/RT-QuIC is an effective biomarker for synucleinopathy with excellent reproducibility.

AP-02-5 Observation of human hand motor control using simultaneous brain and spinal cord functional MRI

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【目的】ヒトの手指巧緻運動は一次運動野から脊髄運動ニューロンへの単シナプス結合（直接運動伝導路）を動員する。最近、サルの損傷研究により手指運動に一次運動野と脊髄運動ニューロン間の多シナプス結合（間接運動伝導路）が参加することが示され、ヒトでの脳・脊髄損傷に伴う麻痺の機能回復過程を考察する上で重要な知見となっている。我々はこの研究からヒントを得て、指先で摘む動作と手指全体で把持する動作で運動伝導路の動員に違いがあると仮説を立て、脳脊髄回路の動員の違いを脳・脊髄同時記録機能的MRI（cs-fMRI）技術で調べた。【方法】右利きの健常人10名を対象とし、利き手、非利き手を用いて母指と示指の指先で摘む動作（指先ピンチ課題）、母指と示指全体で把持する動作（手指把持課題）を行った際の脳および脊髄の活動をcs-fMRI技術を用いて描出した。SPM, FSL, spinal cord toolboxで前処理を行った上で群間比較を行い、uncorrected p < 0.05を有意とした。【結果】指先ピンチ課題において、利き手、非利き手運動ともに反対側の一次運動野および同側のC7-Th1椎体レベルの髄内の神経活動が上昇していた。さらに、非利き手でのみC3-4レベルの髄内の神経活動が上昇していた。手指把持課題同様に利き手、非利き手ともに反対側の一次運動野と同側のC7-Th1レベルの髄内の神経活動が上昇していたが、指先ピンチ課題と異なり利き手、非利き手の運動の双方でC3-4レベルの髄内の神経活動が上昇していた。【結論】サルにおいて間接運動伝導路の中継神経核である固有脊髄ニューロンがC3-4に位置していることから、ヒトにおいてもサルと同様に手指全体を把持する動作で関節運動伝導路の動員を反映している可能性が示唆された。今回の結果は、ヒトの脳・脊髄損傷からの機能回復の機序を考察する上で重要な情報を提供すると考える。

AP-02-6 Genetical and clinical features in a cohort of Japanese patients with dystonia

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【目的】本邦におけるジストニアの遺伝的・臨床的特徴を明らかにする。【方法】Japan Dystonia Consortiumで集積したデータ・サンプルのうち、ジストニアが主徴であり、かつエクソーム解析を行った414例（全て発端者）からなる部分コホートを解析した。エクソーム解析では、DYTナンバーを持つものと、NBIAなどのジストニアを主徴としうる疾患の原因遺伝子に存在する病原性の高いバリアントを検索した。（検索対象遺伝子：TOR1A、HPCA、TUBB4A、GCH1、THAP1、MRI、PRRT2、SGCE、ATP1A3、PRKRA、SLC2A1、CIZ1、ANO3、GNAL、KCTD17、COL6A3、KMT2B、VPS16、AOPEP、VPS11、TH、ADCY5、GNAO1、IRF2BPL、MECR、DNAJC12、ATM、TIMM8A、DCAF17、REPS1、CRAT、COASY、C19orf12、FTL、PANK2、PLA2G6、WDR45）【結果】部分コホートの罹患部位は、focal:169例、segmental:58例、multifocal:10例、hemidystonia:10例、generalized:125例、unknown/unspecified:27例であった。またこの部分コホートには、15例のparoxysmal kinesigenic dystonia、12例のドパ反応性ジストニアが含まれていた。201例でジストニアの家族歴がみられた。エクソーム解析では、TOR1A、TUBB4A、GCH1、THAP1、PRRT2、SGCE、ATP1A3、ANO3、GNAL、KMT2B、VPS16、AOPEP、ADCY5、IRF2BPL、ATM、PANK2に既知の変異や新規かつ病原性が高いバリアントを認めた。【結論】今回解析した部分コホートの大部分は、臨床情報からターゲットシークエンスを行ったが診断が確定しなかった例である。GCH1、PRRT2、SGCEやATP1A3など、一部のジストニア原因遺伝子はかなりspecificな表現型をとる傾向にある一方で、例えばTHAP1、ANO3やGNALなどの表現型はnonspecificであり、現時点では臨床型から原因遺伝子を予測することは困難である。日本におけるジストニアの遺伝・臨床情報を今後さらに集積し、より効果的で精緻なジストニアの診断を可能にすることを目指す。

APe-01-1 Amelioration of HD-associated Phenotypes by Chemical Interference of SUPT4H/SUPT5H Complex Formation

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Objective: Huntington's disease is an autosomal dominant neurodegenerative disorder caused by CAG repeat expansion in the coding sequence of huntingtin gene. As monogenic disorder, the abundance of mutant gene product is the primary determinant for the onset and progression of HD. Accumulated evidence also suggest that mutant HTT suppression could mitigate or prevent the HD pathological development. In our earlier studies, we showed the transcription elongation complex SUPT4H/SUPT5H is required for RNA polymerase II transcribing over DNA region containing a long stretch of CAG repeats. Also, genetic knockdown of SUPT4H results in a decrease of mHTT expression and an alleviation of motor function deficits in a HD mouse model. **Method:** By performing high throughput screening of small molecule libraries using two independent cell-based reporter assays, we have identified a nucleoside compound enabling to interfere with the complex formation of SUPT4H/SUPT5H. **Results:** We demonstrated the compound can inhibit the expression of mHTT gene in a variety of HD cultured cell models, including proliferative murine striatal neuronal cells and terminally differentiated GABAergic neurons. On top of this, in GABAergic neurons, an increased sensitivity to oxidative stress caused by mHTT is reversed by the nucleoside compound. Moreover, alleviation of HD neurodegenerative phenotypes is observed in a *Drosophila* model of HD. **Conclusion:** Our findings suggest that the SUPT4H/SUPT5H protein complex is a potential therapeutic target to lower mutant HTT expression and prevent HD progression.

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APe-01-6 Effects of 40 Hz high-definition tACS on subjective sleep quality and cognition in preclinical AD

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Objective: To investigate the effects of gamma-band (40 Hz) high-definition transcranial alternating current stimulation (HD-tACS) on subjective sleep quality and domain-specific cognitive function in patients with mild neurocognitive disorder due to Alzheimer's disease (NCD-AD). Methods: This study was a double blind, sham-controlled randomized clinical trial. Fifty mild NCD-AD patients were randomly assigned to receive a 4-week course treatment of either HD-tACS, or HD-transcranial direct current stimulation (HD-tDCS), or sham HD-tDCS. Global cognition was assessed by Montreal Cognitive Assessment (MoCA). Subjective sleep quality was assessed by Pittsburgh Sleep Quality Index (PSQI). PSQI total score>5 was defined as sleep disturbances. Results: The mean score of PSQI was 11.7 in mild NCD-AD patients. Repeated sessions of 40 Hz HD-tACS and HD-tDCS treatments significantly enhanced the subjective sleep quality and cognition. Compared with tDCS, the individuals who received 40 Hz HD-tACS had more improvement on sleep quality (score changes of PSQI: 6.01 vs. 3.72, P<0.001) and cognitive function (MoCA: 2.5 vs 1.56) during a 2-month follow-up period. Conclusions: Mild NCD-AD patients with sleep disturbances who received 40 Hz HD-tACS had pronounced enhancement in subjcetive sleep quality and global cognition. To identify novel modality of brain stimulation is important toward an effective strategy for comorbidities in preclinical AD.

APe-01-7 Pons hyperintensity is associated with ischemic damage of middle cerebellar peduncle in CADASIL

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Objective: Autosomal dominant cerebral arteriopathy with subcortical infarcts and leukoencephalopathy（CADASIL）is an inherited cerebral small vessel disease caused by Notch3 mutation. Though hyperintensities trisecting the pons lead to the 'Mercedes-Benz' sign（MBs）and a quadrisection of the pons can lead to a 'hot cross bun' sign（HCBs）. We explored the expression of HCBs and MBs in CADASIL and its relationship with the ischemic damage of the middle cerebellar peduncle（MCP）. Materials and methods: The clinical data of 106 patients with CADASIL confirmed by genetic examination were collected. All patients underwent head MRI. T1 mean, axial T2 and 3D T2 flair were performed on the pons. Diffusion tensor image（DTI）fiber tracking was performed in 18 CADASIL patients and 10 normal controls. Results: MRI of 106 patients showed 12 cases had bilateral MCP lesion and among them, 2 cases had HCBS and 2 cases had Mercedes-Benz sign. The average age of onset of patients with bilateral MCP lesions was 43 years. The median course of disease at the time of MRI examination was 4.5 years. The main clinical manifestations included paralysis, vertigo, neuropsychological disorder, gait disorder and bulbar symptoms. DTI results showed that the mean, radial and axial diffusion ratio of MCP in patients with MCP damage were significantly higher than those in normal controls. Conclusion: Our study confirms for the first time that vascular HCBs and MBs can occur in cerebral small vessel disease, and further confirms that the ischemic damage of bilateral MCP is related to its formation.