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Notes:

- If the abstract will be presented at a Symposium or Workshop, the abstract (Poster)number will be followed by SY (symposium) or WS (workshop) plus the number of the symposium or workshop
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1. Animal models-neurodegeneration

P001

Diabetes-induced expression changes in the trigeminal ganglia of rats: Indicators of inflammation and synaptic plasticity without sign of neuronal injury

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Introduction: Diabetes is a leading cause of neuropathy and can contribute to or exacerbate trigeminal neuralgia through its systemic effects on nerves. Chronic hyperglycemia induces oxidative stress, inflammation, and microvascular damage, leading to nerve injury or dysfunction. In diabetes, elevated pro-inflammatory cytokines may contribute to neuroinflammation in the trigeminal ganglia, enhancing pain signals. Additionally, dysregulation of pain-modulating molecules may amplify nociceptive signaling within the trigeminal nerve**. Objectives and method:** In this study, we performed RT-qPCR and immunohistochemistry to analyze the expression of the neuronal injury marker ATF3, the vasodilator and neurotransmitter Calcitonin Gene-Related Peptide (CGRP), the neurotrophic factor Brain-Derived Neurotrophic Factor (BDNF), the inflammatory mediator Tumor Necrosis Factor (TNF), and the recently identified analgesic target oxytocin receptor (OTR) in the trigeminal ganglia of diabetic, and insulin-treated diabetic rats. **Results and conclusion:** We found that chronic diabetes significantly increased the mRNA expression of BDNF, TNF, and the OTR in the trigeminal ganglia, while levels of CGRP and ATF3 were reduced. These changes indicate potential alterations in neurotrophic and inflammatory pathways, as well as synaptic plasticity, with no evidence of neuronal injury. Interestingly, insulin therapy did not significantly reverse these molecular alterations, suggesting that although insulin can control blood glucose levels, it does not fully address the underlying molecular changes contributing to neuropathy in diabetes. Understanding these factors may help to develop new therapies targeting the complex pathophysiology of diabetic neuropathy, particularly in patients suffering from trigeminal neuralgia. Acknowledgement: Péter Bátor Kemenesi-Gedei was supported by the Hungarian Ministry of Culture and Innovation, National Research, Development and Innovation Fund, EKÖP-KDP-2024. **Keywords**: Diabetes – trigeminal ganglion – neuropathy – synaptic plasticity – inflammation

P002

Single cell analysis of human iPSC-derived neurons carrying the Alzheimer disease-associated APPV717I mutation after long-term engraftment in the mouse forebrain

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Introduction: Alzheimer's disease (AD) is the most common form of dementia affecting millions of people without a cure, and disease mechanisms are still not fully understood. **Objectives:** To gain further insights into disease pathogenesis, we applied a human-to-mouse xenotransplantation approach to assess histological alterations and changes in gene expression in human induced pluripotent stem cell (iPSC)-derived AD neurons in vivo. **Materials & methods**: We differentiated human iPSCs carrying the familial AD APPV717I mutation into neurons, which demonstrated enhanced Aβ42 production, elevated phospho-tau, and impaired neurite outgrowth in vitro. AD and control cells were injected into the forebrain of immunocompromised mice. **Results:** After injection, APPV717I or isogenic control neural progenitor cells differentiated into NeuN-positive neurons representing about 90% of cells in both APPV717I and control grafts at 2 months post injection. 12 months after injection however, APPV717I grafts were significantly smaller and contained an increased number of phospho-tau-positive neurons. We performed comparative single-nucleus RNA-sequencing of microdissected APPV717I and control grafts at 2 and 12 months post injection and found shifts in the cellular composition of grafts with an enrichment of cell death pathways in APPV717I neurons at 12 months post injection, which were not seen in control neurons at that time point or in APPV717I neurons 2 months after injection. **Conclusion:** These data give important insights into transcriptional dysregulation in human APPV717I neurons linked to cellular vulnerability in vivo and provide a unique opportunity to study potentially beneficial effects of therapeutic compounds in this xenograft disease model. **Keywords:** Alzheimer's disease – induced pluripotent stem cells (iPSCs) – disease modeling – neurons – transplantation – single cell analysis

2. Dementia-Alzheimer

P003/WS10
Ageing-related tau astrogliopathy in the oldest old population (Vantaa 85+)

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Introduction: Ageing-related tau astrogliopathy (ARTAG), the occurrence of astrocytic hyperphosphorylated tau, is a common finding in the ageing brain. However, its clinical significance remains elusive and population-based studies are scarce. **Objectives:** To assess the frequency and impact of ARTAG in a general late-life aged population. **Methods:** Immunohistochemical staining using AT8 tau antibody were carried out on the Vantaa 85+ study, a population-based cohort containing over 300 brains of subjects older than 85 years with various levels of cognitive impairment and neurodegenerative pathologies. We semi-quantitatively assessed the appearance of pathognomonic tau deposits, thorny-shaped (TSA) and granular/fuzzy (GFA) astrocytes and their regional distribution in the hippocampus at the level of lateral geniculate body of the oldest old by light microscopy. We further evaluated the association of ARTAG with comorbid pathologies and clinical parameters. **Results:** The presence of hippocampal subependymal TSA (p=0.015), perivascular TSA (p=0.036), solitary (p=0.049) and clustered (p<0.001) grey matter GFA showed an association with male sex. No association with age at death was seen. Subependymal (p=0.035) and perivascular TSA were associated with dementia (p=0.022). Perivascular TSA and Braak stage showed an association (p=0.022). Arteriolosclerosis in the frontal lobe was associated with hippocampal laminar subpial (p=0.002), and white matter (p=0.004) TSA. **Conclusion:** Subependymal and perivascular ARTAG were associated with

a clinical history of dementia in this population-based study on oldest-old, however further research to assess the responsible mechanisms and influential factors is warranted. **Keywords:** Dementia – neurodegeneration – ARTAG – hippocampus – age

P004

Alpha-synuclein distribution patterns relate to different Alzheimer's disease neuropathological phenotypes

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Introduction: While pathological α-synuclein (α-syn) deposits are a common feature in Alzheimer's disease (AD), the relationship with the AD neuropathological phenotype remains elusive. **Objectives:** We aim to quantify the relationships between α-syn, amyloid beta (Aβ) and tau distribution in AD. **Materials & methods**: Therefore, α-syn, Aβ and tau deposits were assessed in 68 AD patients in up to 28 brain regions of specimens from the Munich Brain Bank. A random forest pixel classifier quantified the diaminobenzidine staining for α-syn42, 4G8 and AT8. The AD cases were allocated to subgroups regarding their α-syn distribution approximated by hierarchical clustering. These subgroups were compared in terms of Aβ and tau covered area with a linear mixed effects model across regions and with a Kruskal-Wallis-test and Conover-post-hoc analysis in each brain region, respectively. **Results:** We found four α-syn distribution patterns in AD: (A) negative (43%), (B) amygdala-predominant (18%), (C) brainstem-amygdala-predominant (10%) and (D) disseminated (29%). The subgroups differed significantly regarding their Aβ and tau distribution. Only A and B showed a similar tau distribution while A and C showed a similar Aβ distribution. On a regional level, C showed significantly less tau covered area in cortical regions and the hippocampus compared to the other groups. B and D showed more Aβ covered area in the cortex and hippocampus although not significantly. **Conclusion:** Four distinct AD neuropathological phenotypes were defined using regional distribution differences of α-syn. Such a patient stratification may be beneficial in determining effectiveness of therapies aimed at Aβ or tau. **Keywords:** Alzheimer's disease – alpha-synuclein – immunohistochemistry – digital pathology

P005/WS02

Comparison of neuropathological fibrils from Alzheimer's disease, scrapie and other neurodegenerations using electron cryo-microscopy

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Introduction: The first cryo-EM structure of neuropathological fibrils from Alzheimer's disease was published in 2017. Thereafter, structures from multiple human dementias and motor disorders and recently from scrapie in mice and hamsters have been published. **Objectives:** To carry out a survey of neuropathological fibrils in the Electron Microscopy Data Bank (EMDB), comparing and contrasting features according to the underlying proteins and disease types. **Methods:** The visualization software

UCSF ChimeraX was used as an atomic level “microscope” to compare and contrast features of published fibrils from EMDB. In particular, the size and shape and relationship to protein of a second unknown component was examined, to obtain clues as to its molecular identity. **Results:** Remarkably, regardless of protein or disease type (human vs animal, infectious vs non-infectious), similar structures were found. Fibrils comprised stacks of protein with a second unknown component acting as a supporting rod. **Conclusion:** The features of the second component suggest an anionic polymer, feasibly RNA. Evidence that fibrils from prion and prionoid diseases share a common basic structure suggests that the differences between them may not be as wide as previously suspected. **Keywords:** Neurodegeneration – Alzheimer's dementia scrapie cryo-EM

P006

Decoding GFAP in dementia: A focus on the medial temporal lobe

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Introduction: Astrocytes express GFAP, a marker of astrocytic activation and structural integrity. A recent study by our group links serum GFAP levels to Alzheimer’s disease (AD), suggesting its value as a diagnostic biomarker. **Objective:** Our aim is to examine the histopathological distribution of GFAP and its correlation with neuropathological classification variables. **Materials & methods:** We analyzed 156 brains from the VARS dementia cohort. We developed immunohistochemistry assays for GFAP in entorhinal cortex (EC) and amygdala (A) and measured GFAP area through Cell-Profiler program. **Results**: We focused our analysis on the EC superficial layers and A basolateral nuclei, as they displayed the highest burden of astrocytosis. We found a correlation of tissue GFAP with age at onset for EC and A ($r=-0.291$, $p<0.001$ and $r=-0.234$, $p<0.01$, respectively). In EC, with GFAP in serum ($r=0.211$, $p<0.05$), time of survival ($r=0.217$, $p<0.01$) and brain weight ($r=-0.197$, $p< 0.05$). In A, with age at exitus ($r=-0.209$, $p<0.05$), and LPC classification ($r=0.194$; $p<0.05$). Additionally, GFAP in EC showed a trend of increasing staining with higher stages of Braak tau and TDP43; while both in A and EC, a trend was observed with Braak α-syn stages. Finally, the condition with the highest staining of GFAP in EC was AD while in A it was LBP. The most prevalent neuropathological groups were AD (28.74%), AD+LATE (15.57%), and AD+ VD+LATE (8.98%). **Conclusion:** Our study shows that GFAP immunoreactivity in EC and A correlates with neuropathological variables, supporting GFAP's potential as a diagnostic biomarker for AD and related pathologies. **Keywords:** Astrocytosis - GFAP – Alzheimer's disease & Medial temporal lobe

P007

Exploring patterns of cerebellar amyloid angiopathy

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Introduction and objective: To compare patterns of cerebral amyloid angiopathy (CAA) in the cerebrum vs cerebellum. **Materials and methods:** 24 sequential neurodegenerative autopsy brains

with a dementia clinical diagnosis, 12 males and 12 females, ages 56-93 (average 78), and neuropathologic diagnoses of Alzheimer's disease (21), cerebrovascular disease (20), TDP43opathy (11), LBD (8), PART (1), and CADASIL (1). Meningeal, parenchymal, and capillary CAA plus amyloid plaques were scored 0-3 in frontal, occipital and temporal cortices, plus cerebellum, using beta-amyloid immunohistochemistry. Clinical records reviewed and vascular anatomy assessed using pre-mortem imaging and/or macroscopic neuropathology images. **Results** CAA was identified in 21/24. Cerebellar CAA present in 17/24, never without some CAA in one neocortical region. The cerebellar CAA score exceeded the score in any neocortical region in 4, was equal in 9, and less than neocortical in 7. In the cerebellum meningeal exceeded parenchymal scores in 12, and parenchymal exceeded meningeal in 5. Cerebellar CAA with scores up to 3 was present in cases without cerebellar plaques; likewise cerebellar plaque scores of 2 were present in a case without cerebellar CAA. There was never capillary CAA in the absence of parenchymal CAA in any region. There was no correlation between meningeal and parenchymal CAA scores in the cerebellum, whereas they were highly correlated in neocortical areas. **Conclusion** Cerebellar CAA is common and often equal or more severe than neocortical CAA. In the cerebellum, unlike the neocortex, meningeal and parenchymal CAA are not correlated, suggesting they may be preferentially driven by separate mechanisms. **Keywords:** amyloid angiopathy – cerebellar

P008

In advanced age dementia patients, hippocampal sclerosis without LATE display predominantly early stages of HS and low vascular scores

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Introduction: In advanced age and neurodegenerative dementia, hippocampal sclerosis (HS) and limbic associated TDP-43 encephalopathy (LATE) are closely associated entities. However, their possible pathogenic relationship is under discussion. **Objectives**: Our objective is comparing HS cases with or without associated LATE in a series of postmortem brains from advanced age patients with dementia. **Materials & methods:** We analyzed 210 dementia cases from the BT-CIEN brain bank cohorts (mean age at death= 82.8+/-10.7; females = 69.5%; 83.2% with Alzheimer's disease [AD] as main neuropathological diagnosis, including 29.1% of early-onset AD (EOAD)). All cases underwent full neuropathological work-up limited to the left hemibrain, including a HS staging scheme developed by our group. Brains with HS-like hippocampal microinfarcts were identified and excluded as HS. **Results:** HS was more prevalent among EOAD brains than in late onset AD (LOAD) cases (Chi-square, $p<0.01$). From the 141 EH+ cases identified (including early stages), 23 brains were LATE(-) (16,3%). This group displayed a significantly lower age at death and survival time, and a higher brain weight than the LATE(+) group (Mann-Whitney, $p<0.05$). LATE(-) HS(+) brains showed also lower vascular scores, presented predominantly early HS stages (Mann-Whitney, $p<0.01$), and a lower prevalence of ARTAG (Mantel-Haenzel, $p<0.001$). No significant differences were observed between LOAD and EOAD cases, and no association with APOE e4 haplotype was observed. **Conclusions:** TDP-43(-) HS is more prevalent in early HS. LATE could be a driver for advanced (full) HS in cases with early stages of sclerosis, together with microvascular and ARTAG combined pathologies. **Keywords:** Hippocampal sclerosis – LATE – ARTAG – LOAD – EOAD

P009

Increased and sustained expression of Bax and Survivin is seen in human post-mortem tissue following traumatic brain injury

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Background. Wnt signaling pathway is a complex system that regulates cell growth, tissue repair and neurogenesis, as well as influencing apoptosis and neuroinflammation. The Wnt pathway has been shown to be affected after traumatic brain injury (TBI) in animal models and may underlie the long-term response and increased susceptibility to neurodegeneration seen after TBI. **Materials & methods.** The study we examined the expression of Survivin, Bax, and Bcl-2 (key interactors of the Wnt pathway) in acute and chronic stages of TBI in post-mortem human brain tissue from 12 cases. All donors had sustained contusion injuries to the frontal or temporal lobes, 6 cases showed acute injury (survival <24hr) and 6 cases chronic injury (24hr-16yr survival). Age-matched controls were also included. Immunohistochemistry was performed for Survivin, Bax and Bcl-2 on fixed sections from the lesion site and analyzed using a semi-quantitative scoring system. **Results.** Significantly increased expression of Survivin and Bax in perilesional and pericontusional regions was found (Two-Way ANOVA with Kruskal-Wallis Test ($p<0.05$)), evident early as 15 minutes post injury and continuing to be expressed up to 16 years after insult. In contrast, no immunoreactivity for Bcl-2 was seen in either acute or chronic TBI. **Conclusion.** Findings strengthen the concept of TBI as a continuum rather than a single event and this study provides important evidence of Wnt pathway component's expression in human TBI tissue with under 2 hours survival and in longer survival cases. Understanding the long-term response to TBI may reveal new pharmacological innovations for clinical management of TBI.
Keywords: Neurodegeneration – traumatic brain injury – neuroinflammation – survivin – Bax

P010/WS10

LATE-NC is one of the most significant determinants of dementia in the very elderly - the population-based Vantaa 85+ study

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Introduction: Population-based cohort studies play a crucial role in unravelling the underlying causes of dementia among elderly. The frequency of limbic-predominant age-related TDP-43 encephalopathy neuropathological change (LATE-NC) has only rarely been studied within a population-based context. **Objectives:** We examined the frequency of LATE-NC and its correlations with other brain pathologies and cognition in the population-based Vantaa 85+ cohort. **Methods:** The Vantaa 85+ cohort consists of 601 individuals aged >85 years residing in Vantaa, Finland in 1991. Neuropathological assessments were conducted on 304 subjects (51%), with LATE-NC staging feasible for 295. LATE-NC staging relied on TDP-43 immunohistochemistry, following recently updated guidelines. Standard statistical methods were employed to analyze associations between LATE-NC and other neuropathological variables (such

as Alzheimer's disease neuropathological change, Lewy-related pathology, hippocampal sclerosis, arteriolosclerosis), and cognitive metrics. **Results:** Among the 295 subjects, LATE-NC was present in 189 (64%), with stage 2 being the most prevalent (29%) followed by stage 3 (13%), while stages 1a, 1b, and 1c were less frequent (10%, 5%, and 8%). Stages 1a (P< 0.01), 2 (P< 0.001), and 3 (P< 0.001) were significantly associated with dementia and lower MMSE scores. LATE-NC exhibited associations with Alzheimer's disease neuropathological change (P< 0.001), hippocampal sclerosis (P< 0.001), diffuse neocortical Lewy-related pathology type (P< 0.001), and amygdala arteriolosclerosis (P< 0.02). Across all six multivariate models, LATE-NC emerged as one of the most robust independent predictors of dementia. **Conclusion:** This population-based study underscores the significant role of LATE-NC as an independent determinant of dementia within the general late-life population. **Keywords:** LATE-NC – dementia – oldest-old – population-based – 85+

P011/WS10

Medial temporal lobe pathologies: Interactions, relation to APOE, and their role in hippocampal degeneration

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Introduction: The hippocampus is among the earliest brain regions affected in dementia-related neurodegenerative diseases. While Alzheimer's disease (AD) has traditionally been seen as the primary cause of hippocampal neuronal loss, other lesions, such as limbic-predominant age-related TDP-43 encephalopathy neuropathologic changes (LATE-NC), also contribute. Age-related lesions and the APOE ε4 allele, a major genetic risk factor for dementia, show pathogenic synergy, complicating the clinicopathological picture. **Objectives** Examine the associations between medial temporal lobe pathologies, hippocampal damage, and the APOE ε4 allele in older population. **Methods:** We analyzed post-mortem brain tissue from 480 cases (ages at death 50–99). Using over 30,000 manually annotated neurons from the posterior CA1, we trained an algorithm to automatically quantify neuronal density across the cohort. We also examined brain weight, cognitive status, and the spread of lesions, including Amyloid-β, tau, LATE-NC, α-synuclein, cerebral amyloid angiopathy (CAA), vascular lesions, and Hirano bodies, and performed APOE genotyping. **Results:** We found that, in addition to AD pathology, LATE-NC, amygdala-predominant α-synuclein, small vessel disease, and atherosclerosis are related to CA1 degeneration, while Hirano bodies seem protective against it. Structural equation modelling revealed a complex and strongly interconnected network of common pathologies, showing that tau, LATE-NC, and α-synuclein drive hippocampal neuronal loss, and that Amyloid-β, CAA, and TDP-43, but not tau, are directly influenced by APOE ε4. **Conclusion:** Hippocampal damage in age-related dementias is multifactorial, extending beyond Aβ and tau, with LATE-NC playing a major role. APOE ε4 contributes indirectly via Aβ-related pathogenesis, triggering interactions among comorbid pathologies driving neuronal loss. **Keywords:** Hippocampus – digital pathology – Amyloid-β – α-synuclein – LATE – APOE ε4

P012

Revisiting the role of white matter ARTAG in language deficits in Alzheimer's disease

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Introduction: White matter tau-positive thorny-shaped or granular-fuzzy astrocytes (WM-TSA/GFA), part of ARTAG lesions, have been linked to language changes or focal presentation in Alzheimer's disease in some studies, while others found no such association. **Objectives:** We aimed to evaluate whether the presence of WM-TSA/GFA in the language areas was associated with language symptoms, in an Alzheimer's disease clinicopathological cohort. **Materials & methods:** In 17 cases, AT8/tau-stained slides from the left hemisphere anterior Broca and Wernicke areas were evaluated for the presence and density of WM-TSA/GFA. Total tau cortical and white-matter pathology, neurofibrillary tangles (NFT) and neuritic plaques (NP) were evaluated using semiquantitive methods. Clinical and sociodemographic data were reviewed, and cases were dichotomized according to whether language impairment was an early reported sign (LPos vs LNeg). Statistical studies were done using SPSS software. **Results:** WM-TSA/GFA were identified in 2/9 LPos cases (22,2%) and in 6/8 LNeg cases (75%). Proportions were comparable in Broca and Wernicke areas. There were no significant differences in WM-TSA/GFA density between LPos and LNeg cases (p=0,055). Age at death was found to be significantly lower in LPos group (p=0,046; median 62vs70years), with no differences in age of onset or disease duration. Total tau cortical or white-matter pathology, NFT or NP burden were not significantly different between LPos and LNeg cases nor correlated with WM-TSA/GFA. **Conclusion:** In our AD cohort, WM-ARTAG in the language cortices was not associated with language symptoms. We found a non-significant tendency towards the opposite association, which might be clarified with a larger and better clinically characterized cohort. **Keywords:** Alzheimer's disease – ARTAG – language – white matter – astrocytes – tau pathology

P013

TDP43 pathology in an early-onset Alzheimer´s disease cohort

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Introduction: TDP-43 pathology increases with age and is common in Alzheimer´s disease (AD). The frequency and distribution in sporadic early-onset Alzheimer's disease (EOAD) are less well characterized. **Objectives:** To evaluate the frequency and distribution of TDP-43 pathology in a cohort of EOAD patients from the Portuguese Brain Bank. **Materials & methods:** pTDP-43-stained slides from the regions according to Josephs et al. stages were evaluated for the presence of TDP-43 pathology. Tau Braak stages, Lewy body pathology (LBP) and cerebrovascular lesions (CVD), as well as clinical and sociodemographic data were collected. Statistical studies were done using SPSS software. **Results:** Twenty-two cases were identified, (5F:17M). Age at onset was 56.9 ± 4.7 (mean ± SD), age at death 66.2 ± 6.1 and disease duration 9.3 ± 4.7. Fifteen cases 68%) had TDP-43 pathology. According to Josephs stages: 8 stage 1 (36,4%), 1 stage 2 (4.5%), 3 stage 4 (13.6%) and 2 stage 6 (9.1%). The two

cases with stage 6 did not show pathology on midbrain. Eleven cases (50%) had LBP and there was no significant CVD. The presence of TDP-43 pathology was associated with worse MMSE score at first visit (r=-0.702; p=0.016), with no association to disease duration or presence of LBP. **Conclusion:** We found a high frequency of TDP-43 pathology and an association with a cognitive measure despite the reduced number of cases. The two cases with frontal cortex pathology skipped pathology in region 5 of the classification. Further studies with a higher number of cases and clinical detailed data are needed to clarify this issue. **Keywords:** TDP-43 pathology – early onset Alzheimer´s disease – LATE – cognition

P014

The Down Syndrome Biobank Consortium: A perspective

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Introduction: Individuals with Down syndrome (DS) are at increased risk for early-onset Alzheimer's disease (AD)- dementia. There is a knowledge gap regarding the underlying neurobiological mechanisms of DS-related AD (DS-AD), partly due to limited access to well-characterized brain tissue and biofluids. **Objectives:** Promote the existence and value of the Down Syndrome Biobank Consortium (DSBC) to the field of DS research. **Patients & methods:** Describe an international consortium of brain banks focused on collecting and distributing brain tissue from individuals with DS. **Results:** DSBC includes 11 biobanking sites in the USA, Europe, UK and India, which have more than 100 DS cases and 150 controls. It is an international network promoting the donation, collection and distribution of DS brain, serum, plasma, and CSF to investigate neurobiological mechanisms and novel biomarkers to fight dementia in DS. The DSBC has standardized tissue collection protocols including minimum recommended brain areas to sample, storage and neuropathologic criteria. DSBC members promote sample sharing and use by external researchers and perform educational activities related to DS research. DSBC members evaluate tissue requests, especially as each collaborating institution may have specific local legal requirements. **Conclusion:** The DSBC provides a framework to collect high quality samples from well-characterized DS and control cases and increase DS tissue accessibility for DS researchers at international level. It hopes to increase collaborative investigations and raise

awareness of the importance of research using postmortem tissue from people with DS, thereby increasing the quality and quantity of scientific publications and grant applications in DS. Join in! **Keywords:** Brain banking – international consortium – down syndrome – Alzheimer's disease – collaborative network – tissue requests

P015/SY08

The impact of limbic-predominant, age-related TDP-43 encephalopathy neuropathological changes (LATE-NC) on the proteome in Alzheimer's disease

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Introduction: Alzheimer's disease (AD) is the leading cause of dementia, characterized by amyloid β plaques and hyperphosphorylated tau-containing neurofibrillary tangles. However, AD exhibits substantial molecular heterogeneity, including various co-pathologies that complicate diagnosis and treatment. Emerging evidence suggests that TDP-43 co-pathology, now known as limbic-predominant age-related TDP-43 encephalopathy neuropathological changes (LATE-NC), plays a significant role in AD progression. **Objectives**: This study investigates the impact of TDP-43 proteinopathy on the AD proteome using liquid chromatography-tandem mass spectrometry (LC-MS/MS) to identify markers associated with AD with LATE-NC co-morbidity. **Materials & methods**: A cohort of 80 well-characterized post-mortem brains was analyzed from two regions: the perirhinal and frontal cortex. Samples underwent serial centrifugations to obtain four distinct protein fractions: soluble, dispersible (insoluble), SDS (insoluble membrane-associated), and formic acid (fibrillar/aggregated). Samples were analyzed using a label-free protein quantification workflow with Zeno SWATH data-independent acquisition (DIA). Differential expression and weighted gene correlation network analysis (WGCNA) were utilized to identify proteins altered in AD cases with coexisting LATE-NC compared to AD cases without LATE-NC. **Results:** Preliminary data revealed >900 differentially expressed ($p<0.05$) proteins and 24 WGCNA modules associated with AD+LATE-NC ($p<0.05$) across all four protein fractions. Gene ontology analysis indicated the enrichment of proteins related to synaptic degeneration, ubiquitination, and autophagy, with altered protein abundance levels of those molecular pathways associated with AD+LATE-NC. **Conclusion:** These preliminary results suggest that LATE-NC exacerbates the neurodegenerative process in AD by impairing molecular pathways relevant to neuronal survival. These findings highlight the role of LATE-NC in AD pathogenesis. **Keywords:** Alzheimer's disease – limbic-predominant – age-related TDP-43 encephalopathy neuropathological changes (LATE-NC) – Mass Spectrometry

P147

Astrocyte analysis in Alzheimer disease: Digital pathology insights across key brain regions and co-existing neuropathologic changes

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Introduction: Alzheimer's disease (AD) is defined neuropathologically by the presence of amyloid-beta plaques and tau neurofibrillary tangles. Glial fibrillary acidic protein (GFAP) is an astrocyte cytoskeleton component involved in cell-to-cell communication and blood-brain barrier maintenance. Previous studies have examined reactive astrocyte transformation in neuropathologic degeneration caused by AD. **Objectives:** Few studies have explored differences in astrocyte counts between Middle Frontal Cortex (MFC) and Hippocampal human brain regions encompassing the entire spectrum of AD neuropathologic change (ADNC). **Materials & methods:** Immunohistochemistry was performed on postmortem MFC (n=48) and hippocampal (n=56) brain regions. Co-existing pathologies were included in initial regional comparison and excluded for specific group comparisons. Slides were immunostained with an antibody against GFAP. HALO, a digital pathology software, was used to analyze each slide according to a quantitative percentage positivity threshold (weak=0.07, moderate=0.18, strong=0.27). **Results:** Analysis of GFAP-stained astrocytes across brain regions showed variability between the percentage of positively stained tissue. No differences in GFAP percent positivity were identified in TDP+ vs. TDP- cases when matching for ADNC high in the hippocampus when excluding co-existing pathologies (p=0.14). When assessing all TDP+ cases, hippocampal GFAP % positivity was increased with ADNC low vs ADNC high (p=0.03) but not in the MFC (p=0.67). **Conclusion:** Results reveal differences in astrocyte percent positivity between brain regions, with significant differences in TDP+ cases based on ADNC. These findings suggest that region-specific astrocytic responses to ADNC severity in TDP+ cases may influence disease progression, highlighting their potential as biomarkers or therapeutic targets. Future studies with larger cohorts are needed to expand these findings. **Keywords:** Alzheimer's disease – amyloid-beta – tau – digital pathology

3. Epilepsy

P016

Does mitochondrial dysfunction play a role in pathogenesis of mesial temporal lobe epilepsy secondary to hippocampal sclerosis?

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Background and objective: Studies in animal models of temporal lobe epilepsy suggest a pathogenetic role for mitochondrial dysfunction, although validation studies in humans are scarce. We chose to evaluate the mitochondrial status in the hippocampus resected from patients with mesial temporal lobe epilepsy (MTLE) through proteomic approaches. **Materials and methods**: Crude mitochondrial preparations from human hippocampus samples, resected from patients with MTLE, who underwent amygdalohippocampectomy (Early-onset <10years of age, n=9 and late-onset >11years of age, n=9), compared with age matched normal controls (n = 9) were subjected to quantitative proteomics using high-resolution mass spectrometry (MS). MS data was validated by mitochondrial respiratory chain complex assays (CI- CIV). **Results:** The MS identified 7,961 proteins among which, 190 proteins and 60 mitochondrial proteins differentially over expressed in early and late onset respectively ($p<0.05$). Proteins associated with biological processes such as mitochondrial electron transport chain, mitochondrial translation and branched-chain amino acid catabolic process were differentially overexpressed in cases with early onset MTLE, suggesting a pathogenetic role. Fatty acid beta-oxidation and glutathione metabolic processes were common to both early and late onset MTLE. Mitochondrial respiratory complex activity was higher in early onset compared to late onset MTLE and controls, validating the proteomics data. The activities of mitochondrial complexes II-IV remained unaltered. **Conclusions and clinical relevance:** Mitochondrial dysfunction in hippocampus appears to have a role in the pathogenesis of early onset MTLE, in particular, mitochondrial complex I subunits. Evidence for the role of mitochondrial dysfunction may aid in the development of novel therapeutic strategies for treatment of MTLE. **Keywords:** Hippocampal sclerosis (HS) – age at onset of epilepsy – mitochondrial dysfunction – mass spectrometry – mitochondrial proteomics

P017/SY03

Impact of SLC35A2 variants on protein expression in mild malformation of cortical development with oligodendroglial hyperplasia in epilepsy (MOGHE)

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Introduction: Mild malformation of cortical development with oligodendroglial hyperplasia in epilepsy (MOGHE) is a neuropathological entity associated with drug-resistant epilepsy, affecting mainly young individuals, and primarily localized in the frontal lobe. Approximately 50% of patients harbor a brain somatic variant within the X-linked *SLC35A2* gene, encoding a UDP-Galactose (UDP-Gal) transporter. Preliminary clinical trials of oral D-galactose supplementation have shown clinical improvement in patients with MOGHE harboring *SLC35A2* mosaicism. **Objectives**: This study aimed to evaluate the impact of *SLC35A2* variants on protein expression in brain tissue from patients with MOGHE, with and without *SLC35A2* mosaicism, and distinguishing missense from nonsense variants. **Methods**: Brain tissue samples from 59 genetically tested patients with MOGHE were analyzed using immunofluorescence (IF) and Western blot (WB). The cohort included 13 patients with *SLC35A2* missense variants and 15 with nonsense variants, with variant allele frequencies (VAFs) ranging from 3% to 52%. **Results**: The main protein allocation was observed in perinuclear areas and co-localized

with CNPAse, OLIG2, 58k, TPPP, and BCAS1. Protein distribution was significantly altered in tissue samples with MOGHE, depending on the *SLC35A2* variant type and VAF. IF and WB analyses revealed a marked reduction in SLC35A2 protein levels in tissues with nonsense variants, whereas missense variant samples showed protein redistribution, characterized by apparent accumulation in perinuclear areas. **Conclusion**: Our findings demonstrate that *SLC35A2* variants significantly affect protein expression in MOGHE, with nonsense variants resulting in reduced protein levels. Furthermore, the variant type may influence therapeutic responses to targeted treatments, such as oral D-galactose supplementation. **Keywords:** MOGHE – neuropathology – SLC35A2 – oligodendrocytes – epilepsy

P018

Neuroprotective and multi-target modulation of tonifying and tranquilizing herbs in drug-resistant epilepsy: Network analysis

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Introduction: Drug-resistant epilepsy (DRE) remains a major clinical challenge, affecting approximately one-third of epilepsy patients. Conventional antiepileptic drugs (AEDs) primarily target ion channels to suppress seizures but do not address neuroinflammation, oxidative stress, or neuronal degeneration, which contribute to disease progression. This study explores the neuroprotective and multi-target therapeutic mechanisms of tonifying and tranquilizing herbs used in traditional epilepsy treatments. **Materials & methods:** We analyzed 159 epilepsy-related formulas from nine historical manuscripts using Netminer 4.5 and identified 143 herbal components. Clustering analysis revealed a distinct tonifying and tranquilizing group (Group 3), including Ginseng Radix, Polygalae Radix, Acori Graminei Rhizoma, Angelicae Gigantis Radix, and Cnidii Rhizoma. Their neuroprotective effects were examined through pharmacological data, focusing on their impact on the nervous system. **Results:** Group 3 herbs exhibited significant neuroprotective and neurogenic properties, distinct from AEDs. Ginseng Radix reduces oxidative stress and inhibits neuroinflammation. Acori Graminei Rhizoma enhances GABAergic signaling, suppresses glutamate excitotoxicity, and promotes IGF-1 expression, a key factor in neurogenesis. Polygalae Radix increases NMDA receptor activity, supporting synaptic plasticity. Cnidii Rhizoma regulates inflammatory cytokines and modulates the balance between glutamate and GABA. These herbs provide multi-target modulation, contributing to long-term seizure control and neuroprotection in DRE. Chronic seizures in DRE patients contribute to neuronal damage and neuroinflammation. Group 3 herbs, with tonifying and tranquilizing properties, exhibit anti-inflammatory, neuroprotective, and neurogenic effects, supporting long-term seizure control. **Conclusion:** These findings suggest that herbal medicine or derived compounds may serve as alternative neuroprotective therapies for preventing neuronal degeneration in DRE. **Keywords:** Epilepsy – neuroprotection – multi-target therapy – network analysis – herbal medicine

P019/SY15

Spatial transcriptomic analyses in Ganglioglioma tissue reveal immature neuronal niches forming a dysfunctional neuronal network via inflammation-triggered ECM remodeling

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Introduction: Gangliogliomas (GGs) are rare mixed glioneuronal tumors linked to pharmacoresistant focal epilepsy. Although surgical resection often improves seizure control, the mechanisms underlying GG pathogenesis and epileptogenicity are uncertain. The role of the neuronal component, whether preexisting or neoplastically transformed, remains controversial and poses challenges for neuropathological diagnosis and understanding of the epileptogenic phenotype. **Objective**: This study used spatial transcriptomics to examine neuronal markers and differentially regulated signaling pathways in GGs. **Methods**: Spatial transcriptomic data from 10X Visium of eight histopathologically confirmed GG samples were analyzed using SPATA2. Cell type deconvolution (cell2location) and Weighted Gene Correlation Network Analysis (WGCNA) characterized the tumor microenvironment. **Results**: The transcriptional landscape in GGs demonstrated was heterogenous, matching the variable histomorphologic phenotype. Three main transcriptional patterns were identified: (i) immature neuronal niches enriched in progenitor-like genes (*NRP2*, *HOPX*); (ii) extracellular matrix (ECM) remodeling impacting neuronal development, tumor morphology, and homeostasis; and (iii) distinct cellular niches. WGCNA identified eight transcriptional groups covering astrogliosis (*GFAP*, *S100A1*) and oligodendroglial response (*MBP*, *PLP1*). Interestingly, immature neurons (*HOPX*, *GRIA1*) associated with ECM remodeling and immune infiltration, represented by microglia/macrophages (*CD63*, *FGF12*, *S100A10*) and neuroinflammatory response (*CX3CL1*, *CD44*, *MAPK10*), suggesting a tight interaction between immune and tumor cells. Activation of synaptic signaling (*SNAP25*, *SYP*) and plasticity (*ENC1*, *CHGA*, *CHGB*) in neuron-rich groups pointed towards dysfunctional network remodeling. **Conclusion**: Mapping transcriptional patterns in GGs reveals a progenitor-like neuronal population impacted by immune cell infiltration. Our results further suggest that the inflammatory response triggers ECM and neuronal network remodeling, thereby contributing to increased epileptogenicity in GGs. **Keywords**: Ganglioglioma – Epilepsy – Spatial Transcriptomics – Extracellular Matrix

4. Forensic Neuropathology

P065

Bilateral globus pallidus necrosis due to positional asphyxia in an alcohol-intoxicated patient?

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Introduction/background: Bilateral globus pallidus necrosis is commonly associated with metabolic or toxic etiologies, such as carbon monoxide intoxication. However, more recent publications suggest it is a nonspecific consequence of cerebral hypoxic injury from diverse causes. Given the globus pallidus' high demand for glucose and oxygen because of its high metabolic activity, it is particularly susceptible to systemic insults. **Case:** A 56-year-old man with a history of chronic alcohol abuse and diabetes mellitus type 2 was admitted to the hospital after being found comatose (Glasgow Coma Scale: 6/15) in an upright sitting position. Witnesses reported he had remained in this upright position throughout the previous night and day, spanning approximately 20 hours. Laboratory findings indicated hypoxia, acidosis, hyperglycemia, rhabdomyolysis and acute kidney failure. His blood alcohol concentration

(BAC) was 0.3 per mille. Brain MRI revealed bilateral, symmetrical globus pallidus infarctions. Despite intensive care, the patient succumbed 12 days after admission. A forensic investigation was initiated into possible poisoning and/or culpable negligence. Autopsy confirmed symmetrical tissue necrosis confined to the globus pallidus region with macrophage-mediated phagocytosis. Extensive toxicological screening ruled out exogenous intoxication and there was no evidence of methylmalonic acidemia or vascular abnormalities. **Conclusion:** We hypothesize that, given the described circumstances, positional asphyxia in an alcohol-intoxicated state, with an estimated BAC of 3.0 to 3.5 per mille at the time of the incident, may have led to cerebral hypoxia and thus global pallidus necrosis. Additionally, as previously suggested in the literature, the diabetic hyperglycemic state might have been an additional factor of metabolic dysregulation. **Keywords:** Forensic pathology – globus pallidus – necrosis – positional asphyxia – cerebral hypoxia – basal ganglia

P067/WS07

Extracranial hemorrhages in pediatric abusive head trauma: Pathophysiological and forensic considerations

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Introduction: Pediatric abusive head trauma (AHT) is frequently characterized by the triad encephalopathy, intracranial subdural hemorrhage, and multiple retinal hemorrhages. Other pathological findings include fractures, spinal subdural hemorrhages, hypoxic-ischemic/hemorrhagic injuries of the spinal cord, focal hemorrhages in other structures of the orbital cavity. The different aspects of pediatric AHT are strictly anatomical in nature, with regards to pathophysiologic mechanisms and correlated methods of investigation. **Objectives, materials & methods:** Here, we evaluate extracranial hemorrhages in the context of the orbital cavity and vertebral canal, in a case series of AHT. **Results:** In the orbital cavity, subdural hemorrhages at the level of the optic nerve were detected in all cases. Moreover, small hemorrhages in the context of optic nerve, extrinsic eye muscles, and intraorbital adipose tissue were also detected. In the vertebral canal, subdural hemorrhages were detected not only at the cervical level but also in the thoraco-lumbo-sacral segments, where hypoxic-ischemic damages and radicular injuries/hemorrhages were also specifically identified, supporting the pathophysiologic mechanism of direct traumatic damage also for caudal spinal subdural hemorrhages. **Conclusions:** Extracranial hemorrhagic findings represent further significant aspects to be investigated to establish the abusive nature of trauma in forensic contexts. From a methodological point of view, integration of intra vitam/postmortem imaging, for osteo-musculo-ligamentous structures, and exhaustive histopathological study, for anatomo-topographical correlations, is also pivotal. **Keywords:** Abusive head trauma – trauma – forensic neuropathology

P070

Prevalence and location of traumatic axonal injury and vascular axonal injury in fatal traumatic brain injury

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Introduction: Axonal injury (AI) is a frequent manifestation of traumatic brain injury (TBI). The two most common etiologies are traumatic (TAI; due to shearing of axons) and vascular (VAI; due to secondary events such as ischemia). In fatal TBI, a neuropathologist is often consulted to diagnose AI and determine the etiology. **Objectives:** To report the prevalence and location of TAI and VAI in fatal TBI cases. **Materials & methods**: The sample comprised consecutive fatal TBI cases that underwent medico-legal autopsy and in-house neuropathological examination in Helsinki, Finland, in 2023—2024. The neuropathological examination was performed on a formaldehyde-fixed brain, and the diagnosis of AI was based on β-amyloid precursor protein immunohistochemistry. Background characteristics and neuropathology findings were retrospectively reviewed for this report. The statistical approach was descriptive. **Results:** The sample included 21 fatal TBI cases (18 accidents, 1 homicide, 1 suicide, 1 case of undetermined intent; 16 males; age 12—81 years). The median brain weight was 1424 g (range 1017—1694). TAI and VAI were diagnosed in 12 cases each. TAI was most frequently located in the internal capsules (7/12), followed by the genu (6/12) and splenium of corpus callosum (4/12). VAI was most frequently located in the internal capsules (10/12), brainstem (8/12) and splenium (7/12). Other injuries were also present, including subarachnoid hemorrhage in 17 and contusions in 17 cases. **Conclusion:** In this dataset of fatal TBI cases, the prevalence of both TAI and VAI was 57.1%. Internal capsule was the most frequent location of TAI and VAI. **Keywords**: Axonal injury – traumatic brain injury – medico-legal – autopsy

P073

Traumatic brain injury post-mortem donation and 'living' tissue collection from neurosurgical operations – new ventures in brain banking.

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Introduction: Access to donated tissue for research into traumatic brain injury is essential to understanding the molecular characteristics of different types and patterns of Traumatic Brain injury (TBI) and its link to neurodegenerative, psychiatric and neurological diseases. In addition, within neuroscience there is a growing research requirement for surgically resected tissue. **Methods:** Over the last five years, the London Neurodegenerative Diseases Brain Bank has begun two major new initiatives to meet the current needs of the Neuroscience community; post-mortem donation of TBI cases and collection of 'living' tissue from neurosurgical interventions. **Results:** Working alongside Coroners and Forensic Neuropathologists we have collected 92 TBI post-mortem donations with varying survival time (77 acute, 15 chronic) which are comprehensively examined, sampled and classified (including assessment of βAPP, Clusterin, CD68, Tau, βA4 and TDP-43). For living tissue, we have established a successful protocol to collect surgically resected brain tissue excess to diagnostic requirements. Following patient identification and consent, we have obtained tissue from 22 oncology and 32 lobectomies/epilepsy surgeries (with collection success rate of 71% and 98 samples collected). Samples were immediately utilized in research studies or preserved for future use. **Conclusions:** Banking of TBI tissue is essential for research into the acute effects of head injury as well as long-term

consequences and links to neurodegeneration. Surgical tissue has been used in experimental techniques including slice and cell culture, atomic force microscopy, proteomic and transcriptomic analysis, and immunohistology. We aim to continue to increase collection and provision of both in the coming years. **Keywords:** Traumatic brain injury – neurodegeneraton – surgical tissue

P047

Extrapulmonary tuberculosis: A fatal case of cerebellar tuberculosis due to delayed diagnosis

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Introduction: Tuberculosis is rare in Belgium (7.4 per 100,000 inhabitants) but remains a significant infectious disease and one of the top ten global causes of death. Timely diagnosis and treatment are crucial for both patient outcomes and public health. Central nervous system tuberculosis is uncommon, mimics metastatic disease, and is associated with high morbidity and mortality. **Clinical Case:** A 48-year-old Congolese asylum-seeking man with latent tuberculosis presented to the hospital with nausea, vomiting, and weight loss. However, the patient had registered at the hospital under a different identity. Imaging revealed a cerebellar lesion and an enlarged abdominal lymph node, raising suspicion of metastatic disease. A biopsy of the abdominal lymph node was unsuccessful. His condition rapidly deteriorated, and he passed away 12 days later. Due to identity inconsistencies and his asylum status, a forensic autopsy was ordered by the judicial investigator. Histological examination of both the cerebellar lesion and lymph node revealed caseating granulomas with giant cells, and microbiological investigation using a PCR array confirmed Mycobacterium tuberculosis. Cerebellar and lymphatic tuberculosis were diagnosed in the absence of pulmonary lesions. **Conclusion:** This case highlights the importance of early recognition of cerebellar tuberculosis, particularly in at-risk patients with latent tuberculosis. Increased clinical awareness can lead to earlier diagnosis and, consequently, timely treatment, which, while not guaranteeing recovery, can significantly reduce mortality, prevent medical professionals from becoming infected, and improve public health outcomes. **Keywords:** Tuberculosis – central nervous system tuberculosis – cerebellar tuberculosis – forensic autopsy

5. FTD and ALS

P020/WS02

Cystatin C and amyotrophic lateral sclerosis/motor neuron disease

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Introduction: Sporadic MND/ALS is characterized by two pathological inclusions in lower motor neurons. The first, TDP43-positive inclusions, have been extensively studied and are observed in 97% of cases. The second, the Cystatin-C-positive Bunina body (BB), has been relatively overlooked. BBs are accompanied by a reduction in cytoplasmic Cystatin-C. Due to lack of research, and the BB's small size (1-5µm), we hypothesized that this pathology was underestimated. **Objectives:** To assess prevalence

of cystatin C/BB pathology in MND/ALS and to model this in cell culture. **Materials & methods:** Immunohistochemistry on serial sections of postmortem spinal cord and brainstem from individuals in MND/ALS (n=30) and siRNA knockdown of cystatin C in HeLa cells. **Results:** Using more detailed histological analysis than previous reports, we demonstrated that BBs occur within neuronal processes as well as somata. Within our sporadic patient cohort, 100% have somatic BBs, while 96% of cases have depleted cytoplasmic Cystatin-C. TDP43 inclusions only occurred in lower motor neurons with reduced Cystatin-C, demonstrating a relationship between the two pathologies. siRNA-mediated Cystatin-C knockdown, mimicking the cytoplasmic loss in motor neurons, significantly reduces soluble TDP43 levels in vitro and increases cytoplasmic TDP43 accumulation. This appears to increase insoluble 35kDa fragmented TDP43 on immunoblotting. Interestingly, the TDP43 phenotype is worsened, not rescued, when Cystatin-C is overexpressed in these knockdown cells. This overexpression appears to increase a stress phenotype, which is immunopositive for the stress granule marker, G3BP1. **Conclusion:** Cystatin C/Bunina body pathology is present in all cases of sporadic MND/ALS with TDP43 proteinopathy and this likely contributes to TDP43 proteinopathy. **Keywords:** Cystatin C – TDP43 – Bunina body – motor neuron disease – amyotrophic lateral sclerosis

P021

Epigenetic methylation and one carbon metabolism in TDP-43 proteinopathies

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Introduction: Motor Neuron Disease (MND) and Frontotemporal Lobar Degeneration (FTLD) are classed as TDP-43 proteinopathies due to the common presence of TDP-43 proteinopathy within disease-associated CNS regions. Most MND/FTLD patients lack any known monogenic cause, which makes epigenetics and environmental influences particularly important when searching for disease mechanisms. **Objectives:** To investigate the relationship between 1 Carbon metabolism, DNA and histone methylation, and TDP43 pathology in MND/ALS and FTLD-TDP. **Materials & methods:** Immunohistochemistry for TDP43 combined with 5mC and H3K27Me3 on postmortem material form individuals with MND (spinal cord) and FTLD-TDP (hippocampus). Mass spectrometry metabolomic investigation of postmortem brain tissue from individuals with MND and neurologically healthy controls. **Results:** We have used immunohistochemistry in post-mortem MND spinal cord and motor cortex, and FTLD hippocampus to study the association between TDP-43 and two methylation-based epigenetic marks - 5mC (DNA methylation) and H3K27me3 (histone methylation). Using this approach, we have found evidence for dysregulation of these forms of methylation specifically associated with TDP-43 proteinopathy across both diseases. We hypothesized that this may be a result of disruptions within the one-carbon (1C) metabolism pathways, which are responsible for supplying methyl groups for these reactions. To address this, we also conducted a mass spectrometry-based metabolomics study in post-mortem MND brain tissue, and have found evidence for MND-associated dysregulation of the transsulfuration pathway, directly downstream of the methylation reactions. Further immunoblot-based studies appear to show reductions in cystathionine beta synthase, a key enzyme in this pathway. **Conclusion:** TDP43 proteinopathy is associated with loss of DNA and histone methylation in MND and FTLD-TDP. Further, MND is characterized by metabolic aberrations in one carbon metabolism, specifically, the transsulfuration pathway. **Keywords:** TDP43 – motor neuron disease – methylation – one-carbon

P022

Human amyotrophic lateral sclerosis/motor neuron disease: The disease-associated microglial pathway is upregulated while APOE genotype governs risk

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Background: A key role for inflammation in motor neuron disease (MND) has been identified. It is vital to assess which CNS structures are most affected and which inflammatory processes are responsible. **Materials & methods:** The inflammatory transcriptome was characterized in the spinal cord and motor cortex in postmortem human sporadic MND and controls using the nCounter® Neuroinflammation Panel. Archival data were compared with the nCounter data. Immunohistochemistry was used to examine the inflammatory response in the spinal cord, motor cortex, and regions across the brain and transcriptomic analyses. The relationship between APOE genotype and disease risk, survival and age of onset was studied using data from the project MINE database. **Results:** There was marked inflammation in the spinal cord, with less inflammation in the motor cortex. Gene expression analysis in the spinal cord highlighted TREM2, TYROBP, APOE, and CD163 as well as phagocytic pathways. In MND spinal cord, significant microglial reactivity, and involvement of TREM2, ApoE (encoded by APOE) and TYROBP was confirmed, suggesting the involvement of the disease-associated microglial (DAM) phenotype. The corticospinal tracts showed greater inflammation than the ventral horns. The precentral gyrus of ALS/MND again showed less immune reactivity to disease. Finally, in the largest cohort assessed to date, we demonstrate an association between the APOE variant and ALS/MND risk, age of onset and survival. We find associations between APOE ε3/ε3 and disease; and between ε2/ε2 and absence of disease. ε4/ε4 is associated with shorter survival and earlier age of onset. **Conclusion:** While there is widespread inflammation in the CNS in sporadic MND, this is more marked in the spinal cord, especially the corticospinal tract. The DAM phenotype has a key role together with a possible influx of somatic macrophages. APOE ε3 appears to be protective, while ε4 appears to be associated with shorter survival and earlier disease onset. **Keywords:** Motor neuron disease – inflammation – APOE – disease associated microglia

P023/SY14

Morphological changes of microglia in the spinal cord in ALS

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Introduction: Microglia represent up to 15% of cells in the central nervous system and play a fundamental role in neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS). The shape of microglia has been associated with its activity, for example, a branched shape in a state of rest; rounded in an active state; the amoeboid shape with phagocytosis and the rod or dystrophic shapes with age. **Objectives:** To analyze the morphological differences of microglia in the spinal cord

at the level of the anterior horn and corticospinal tract in ALS. **Materials & methods:** Spinal cord sections from 19 patients with ALS and 10 controls without neurological disease were analyzed from autopsies with immunohistochemical staining with IBA1, TMEM119, CD163 and STING1. The quantity, staining surface, cell size, soma perimeter and degree of branching (using Sholl analysis) were studied at the level of the anterior horn and in the corticospinal tract. **Results:** A statistically significant increase in the quantity, size and perimeter of microglia ($p<0.001$) was observed in ALS cases, both in the anterior horn and in the corticospinal tract, compared to controls. Sholl analysis revealed a loss of branch complexity and length in ALS cases. These morphological changes are associated with an increase in CD163 and a decrease in STING1 inflammatory signals. **Conclusions:** We demonstrate quantitative and qualitative changes in microglia in ALS that reflect dysfunction at the level of the spinal cord. Loss of branching complexity may indicate a dysfunctional phenotype that may play a role in disease pathology. **Keywords:** ALS – microglia – IBA1

P024

Neuropathological changes in Parkinson's disease-amyotrophic lateral sclerosis complex (Brait-Fahn-Schwartz disease)

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Introduction: The comorbidity of Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS), termed PD-ALS complex (Brait–Fahn–Schwartz disease), is a very rare entity with lack of precise pathological descriptions in the literature. Here, we report a post-mortem neuropathological examination of a patient with PD and ALS. **Clinical case**: 74-years-old woman showed a 6-year history of progressive paraparesis with dystonic foot position, right-side cogwheel rigidity and bradykinesia with a good response to levodopa. She had also developed proximal-dominant quadriparesis with distal muscle atrophy. The clinical diagnosis of PD and ALS were confirmed in brain MRI, DaTSCAN and EMG. Post-mortem neuropathological examination revealed neuronal loss with astrocyte proliferation in brain cortex, basal ganglia, brain stem nuclei and spinal cord anterior horns, mild demyelination in brain and spinal cord white matter. Immunohistochemical staining showed numerous β-amyloid plaques in brain cortex, hippocampus, basal ganglia (Thal phase 3), numerous tau-positive neurofibrillary tangles, astrocytic plaques/tufts and dystrophic neurites in the brain cortex, hippocampus (Braak stage V), α-synuclein-positive Lewy's bodies and neurites in substantia nigra, locus coeruleus, basal ganglia and brain cortex (Braak stage 6), and numerous TDP-43-positive nuclear and glial cytoplasmic inclusions and dystrophic neurites in medulla oblongata nuclei, spinal cord grey matter and temporal cortex. **Conclusion:** PD-ALS complex shows multiple pathological neurodegenerative changes, including α-synuclein, TDP-43, tau, and β-amyloid pathology. Growing evidence suggests that neurodegenerative diseases may present with overlapping symptoms and share a common pathological basis. **Keywords:** Parkinson's disease – amyotrophic lateral sclerosis – neuropathology – case report – PD-ALS complex

P025

Neuropathological features of two FTLD-MAPT cases with the p.p397s mutation

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Introduction *MAPT* gene mutations cause frontotemporal lobar degeneration with tau inclusions (FTLD-Tau). Sixty-five mutations have been described. **Objectives** Report clinical and neuropathological features of two cases with *MAPT* p.P397S mutation, not reported so far. **Patients & methods** Description of 2 unrelated mutation carriers. **Results** Patient 1 was an 85-year-old male with bvFTD starting at 70. Patient 2 was a 74-year-old male with disease onset at 65. He developed cognitive decline with cortico-subcortical involvement, suggestive of Alzheimer's disease with prominent vascular pathology. Macroscopy showed a fronto-temporal degeneration pattern with severe hippocampal atrophy; case 1 had marked temporal atrophy and case 2 lacunar infarcts in basal ganglia and depigmented s. nigra. Histologically, both cases showed an atypical limbic predominant neuroglial tauopathy with 4R predominant tau isoforms, with preferential involvement of superficial cortical neurons, coiled bodies and granular-fuzzy astrocytes (GFA), ballooned cells, hippocampal sclerosis, limbic TDP43 and amyloid beta (Thal 3, CERAD moderate). Case 1 had marked neuronal involvement of the temporal cortex and fascia dentata and scarce GFAs. Case 2 had less neuronal pathology and abundant GFAs with occasional tufted/ramified morphology. He also showed neocortical Lewy body disease (LBD), cerebrovascular disease and vascular brain injury (CDB-VBI). **Conclusion** The p.P397S *MAPT* mutation leads to a relatively slowly progressive FTD. Neuropathological findings show an atypical 4R tauopathy with prominent limbic involvement that may vary with time progression. While limbic TDP-43 pathology might be related to HS or modulated by the tauopathy, other co-pathologies such as LBD and CVD-VBI may influence the disease course. **Keywords**: FTLD-Tau – FTLD-MAPT – bvFTD – MAPT mutation – Tauopathy – p.P397S

P026

Primary lateral sclerosis as a form of presentation of a sporadic globular glial tauopathy

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Introduction: Globular glial Tauopathy presents with a wide range of clinical phenotypes, making diagnosis challenging. Neuropathological study in these cases is crucial to accurately identify the pathological characteristics that underlie the clinical symptoms. **Objectives:** To describe the clinical and neuropathological findings of two patients with sporadic globular glial tauopathy (GGT) with

clinical presentation as primary lateral sclerosis (PLS). **Methods:** two women began at the age of 73 (patient 1) and 71 (patient 2) with clumsiness and rigidity of the right hand that evolved into an asymmetrical pyramidal motor syndrome, without myoclonus or involvement of the second motor neuron at 12 and 6 years old of evolution respectively. **Results:** In both brains there was severe atrophy of the pre-central motor cortex. Histology findings showed mild superficial spongiosis in layers I and II, marked neuronal loss and gliosis in the precentral gyrus, Betz neurons with signs of degeneration, neuronal loss in the anterior horn of the spinal cord with degeneration of the corticospinal tracts. Immunohistochemistry for hyperphosphorylated tau protein showed: globular neuronal deposits and oligodendroglial tau deposits in the form of globular glial inclusions (GOI) widely present in the prefrontal and motor cortex and especially in the white matter. The diagnosis of GGT type II was established. **Conclusions:** PLS may have GGT as a pathological basis. Whether PLS constitutes a subtype of the amyotrophic lateral sclerosis-motor neuron disease spectrum or a subtype of GGT needs to be confirmed with further prospective clinical and neuropathological studies, as well as new imaging biomarkers. **Keywords:** GGT-Primary lateral sclerosis – motor neuron disease

P027/WS15

Pyroptosis and dipeptide repeat pathology are associated with the same proteome modules in amyotrophic lateral sclerosis (ALS)

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Many genes have been associated with the incidence of ALS, of which chromosome 9 open reading frame 72 (C9ORF72) hexanucleotide repeat expansions are among the most common. However, the majority of cases, classified as sporadic ALS, have an unknown origin. ALS is characterized by neuronal degeneration in the motor cortex, brain stem and spinal cord. Among others, the regulated cell death (RCD) mechanism pyroptosis acts as a potential driver of neurodegeneration in ALS. However, partly due to the heterogeneity of ALS cases, no clear pathway for neurodegeneration has been described in ALS. Moreover, it is unknown whether the rate and type of RCD activation differ between affected regions and ALS subtypes. For this purpose, motor cortex, spinal cord and cerebellar cortex tissue of 15 sporadic ALS, 12 C9ORF72 ALS and 14 control cases was homogenized and serially centrifugated in order to obtain 4 physicochemical fractions. These fractions were analyzed using a label-free protein quantitation workflow with Zeno SWATH data-independent acquisition, followed by subsequent bioinformatic analysis. This analysis was complemented by immunohistochemistry on a subset of human derived ALS tissue. Distinct protein modules in the weighted gene correlation network analysis (WGCNA) were associated with both the number of pyroptosis exhibiting microglial cells and the presence of C9ORF72-related pathology in the spinal cord (Turquoise module Soluble fraction: r=-0.71, p=0.5e-6, Turquoise module Dispersible fraction: r=0.58, p=0.1e-3). These findings indicate that pyroptosis activation plays an important role in the neuroinflammatory response of the neurodegenerative process in ALS, especially in the presence of C9ORF72-related pathology. **Keywords**: Amyotrophic lateral sclerosis-C9ORF72 – Neurodegeneration-Regulated cell death

P028

Small striatal huntingtin inclusions in patients with motor neuron disease with reduced penetrance and intermediate HTT gene expansions

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Introduction: Short tandem repeat expansions in the human genome are overrepresented in a variety of neurological disorders. It was recently shown that huntingtin (HTT) repeat expansions with full penetrance, i.e. 40 or more CAG repeats, which normally cause Huntington's disease, are overrepresented in patients with amyotrophic lateral sclerosis (ALS). Whether patients carrying HTT repeat expansions with reduced penetrance (36–39 CAG repeats), or intermediate penetrance (27–35 CAG repeats), have an increased risk of ALS has not been investigated. **Patients and methods:** We examined the role of HTT repeat expansions in a motor neuron disease (MND) cohort, searched for expanded HTT alleles, and investigated correlations with phenotype and neuropathology. MND patients harboring C9ORF72 hexanucleotide repeat expansions (HREs) were included, to investigate whether HTT repeat expansions were more common in this group. **Results:** We found a high prevalence of intermediate (range 5.63%–6.61%) and reduced penetrance (range 0.57%–0.66%) HTT gene expansions in this cohort compared to other populations of European ancestry, but no differences between the MND cohort and the control cohort were observed, regardless of C9ORF72HRE status. Upon autopsy of three patients with intermediate or reduced penetrance HTT alleles, huntingtin inclusions were observed in the caudate nucleus and frontal lobe, but no significant somatic mosaicism was detected in different parts of the nervous system. **Conclusions:** We demonstrate, for the first time, huntingtin inclusions in individuals with MND and intermediate and reduced penetrance HTT repeat expansions. More clinicopathological investigations are needed to understand the impact of HTT gene expansion-related pleiotropy. **Keywords:** MND – HTT repeat expansions – reduced and intermediate penetrance – huntingtin inclusions

6. Movement disorders

P030/SY04

Alpha-synuclein pathology of the peripheral nervous system in Parkinson's disease: Is it a systemic disease?

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Introduction: The peripheral nervous system (PNS) has recently emerged as a key contributor to Parkinson's Disease (PD) pathophysiology, yet its extent and characteristics remain underexplored. **Objectives:** We investigated alpha-synuclein (αSyn) pathology in vivo in the enteric nervous system (ENS) and skin of PD patients, and ex vivo in peripheral tissues and the CNS of body donors with confirmed alpha-synucleinopathy, using conformation-specific antibodies and Real-Time Quaking Induced Conversion (RT-QuIC). **Patients and methods:** Our in vivo study included 97 PD patients and 28 controls, while ex vivo analysis examined 10 body donors of the Reference Center of the University of Padova. Immunohistochemistry targeted aggregated αSyn (5G4), neuronal and glial markers, immune-cell markers, and nerve fiber proteins, followed by morphometric analysis. **Results:** Aggregated αSyn with a thread-like pattern was detected in the skin of 65% (67/90) of PD patients and in the gut of all biopsied cases (20/20), co-localizing with neuronal markers. Significant quantitative differences between early and advanced PD were observed. Enteric glial cells showed increased size and density, suggesting reactive gliosis. PD patients exhibited increased T- and B-lymphocytes and higher HLA-DR expression in the gut. RT-QuIC accuracy was 87.7% in skin, 67.4% in the duodenum, and 80.0% in gastric biopsies, with higher sensitivity in advanced PD. Ex vivo, αSyn pathology was detected in multiple PNS sites, including the gut, heart, and carotid body. **Conclusion:** We found evidence for αSyn pathology in the PNS, effectively distinguishing PD patients from controls. Further research is needed to determine its early onset and impact on treatment efficacy in advanced PD. **Keywords:** Alpha-synuclein – Parkinson's disease – peripheral nervous system – enteric nervous system – RT-QuIC – immunohistochemistry

P031

Astrocytic processes-dominant α-synuclein accumulation in an autopsy case of multiple system atrophy with over 2 decades clinical course

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Introduction: Multiple system atrophy (MSA) is characterized by neuronal loss with gliosis in the striate-nigral and olivo-ponto-cerebellar structures with phosphorylated α-synuclein accumulation in oligodendrocytes and neurons. Although subpial and subependymal astrocytic α-synuclein accumulation has been reported in cases of MSA with long duration, little has been known regarding cerebral cortical astrocytic α-synuclein pathology in MSA. **Clinical:** A 61-year-old woman at the autopsy developed dizziness and dysarthria at the 38 years of age. She received a diagnosis of MSA-C due to the typical neurological and imaging findings of MSA. Tracheostomy was applied at the age of 48. Voluntary movements disappeared completely at 49 years of age. She died at age 61. The whole clinical course of MSA was 22 years. Neuropathological findings: The brain, weighing 720 g before fixation, showed severe atrophy with discoloration in the brainstem, cerebellum, basal ganglia, cerebral cortex, and cerebral white matter. Severe depigmentation was noted in the substantia nigra and locus coeruleus. Microscopically, severe degeneration was observed in the frontotemporal parts of the cerebral cortices, striatum, pontine nucleus, inferior olivary nuclei, cerebellar white matter, and Purkinje cells. The widespread occurrence of phosphorylated α-synuclein-positive neuronal and oligodendroglial inclusions, such as glial cytoplasmic inclusions, was noteworthy leading to a definite diagnosis of MSA. Regarding astrocytic lesions, astrocyte processes-dominant α-synuclein accumulation in the cerebral cortices was observed in addition to α-synuclein-positive subpial astrocytes in the ventral part of the brainstem. **Conclusions:** Processes-dominant α-synuclein accumulation in the cerebral cortical astrocytes could be observed in MSA with long clinical duration. **Keywords**: Multiple system atrophy – synuclein – astrocyte – processes – long duration

P032/SY08

Cortical layer specific molecular signatures of glial cells in neurodegenerative movement disorders revealed by spatial transcriptomics

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Introduction: Recent studies demonstrate cortical layer-specific molecular signatures of astrocytes based on transcriptomic profiles. How these and other glial cells' molecular profiles are differentially affected in neurodegenerative diseases remains unknown. **Objectives:** To identify cortical layer-specific glial molecular alterations in movement disorders and their potential contributions to pathomechanisms underlying phenotypic diversity and response to diverse conformations of misfolded proteins. **Materials & methods:** The GeoMx spatial transcriptomics platform was used to collect regional cell type-specific transcriptomic signal of astrocytes and microglia across cortical layers and subcortical white matter from the post-mortem human frontal lobe brain samples. Samples included Lewy body diseases (Parkinson's disease (PD) and Parkinson's disease with dementia (PDD), stratified by ApoE genotypes) and progressive supranuclear palsy (PSP) (PSP-Richardson, PSP-corticobasal syndrome, and PSP-parkinsonism). **Results:** We show successful enrichment of glial transcriptomes across all samples, revealing significant differential gene expression between cortical layers. We also show anatomical region-specific differences between phenotypes within the same disease as well as between PD and PSP. **Conclusion:** Some of the identified glial cell-specific gene expression signatures in PD and PSP illuminate potential biological processes and candidate genes that may contribute to phenotypic diversity, with important implications for advancing understanding of disease pathomechanisms, improving disease modelling, and identifying therapeutic targets that limit region- and cell-specific spread of misfolded proteins. Further research is warranted to validate these findings in other brain regions and in larger cohorts, and to investigate the interplay between altered gene expression, genetic and epigenetic modifications, and protein expression in a cell- and region-specific context. **Keywords:** Spatial transcriptomics – Parkinson's disease – progressive supranuclear palsy – movement disorders – glial transcriptomics – neurodegeneration.

P033

Identifying diverse pathways to dementia in Parkinson's disease

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Introduction: Many individuals with Parkinson's disease (PD) develop dementia as the disease progresses. Previous research indicates that factors such as ApoEε4 genotype, combined with

Alzheimer's disease (AD) pathology, or orthostatic hypotension (OH), increase the risk of dementia. However, it remains unclear to what extent these factors explain dementia presence in PD and whether other, yet unidentified factors, could also contribute to dementia development. **Objectives:** To identify distinct pathways leading to dementia in sporadic and genetic PD. **Materials & methods:** Using automated quantitative pathology, we measured the extent of Lewy body, amyloid-β, and pTau pathology across multiple brain regions in over 400 post-mortem PD brain samples. We also examined ischemic vascular pathology and correlated these findings with ApoE genotypes, OH, disease duration, sex, age at death and dementia status. Additionally, we explored the relationship between dementia presence in two distinct Lewy pathology propagation patterns: "body-first" and "brain-first". **Results:** We reveal at least four distinct pathways to dementia in PD patients and our findings suggest that distinct propagation patterns have different risks associated with dementia development. **Conclusion:** The insights from this study have significant translational value, as recognizing different dementia risks in PD patients may improve clinical management strategies. Notably, some PD patients develop dementia without a clear cause, while others with significant misfolded protein pathology remain cognitively intact. Identifying dementia causes and resistance mechanisms may uncover protective factors, highlighting the need for patient-specific clinical management. **Keywords**: Parkinson's disease – dementia – digital pathology

P034/SY04

Iron(ing) out parkinsonisms: exploring the interplay of ferroptosis and proteinopathy in Parkinson's disease and related tauopathies

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Introduction: Parkinsonisms are characterized by motor deficits from dopaminergic neuron degeneration in the substantia nigra (SN). Parkinson's disease (PD) symptoms overlap with tauopathies: Progressive Supranuclear Palsy (PSP) and Corticobasal Degeneration (CBD). Both alpha-synuclein, PD hallmark, and tau, PSP/CBD hallmark, are linked to ferroptotic iron-induced cell death. **Objectives:** Investigate ferroptosis-related changes at (1) protein and (2) transcriptome level in dopaminergic neurons from parkinsonisms and (3) biologically test these outcomes in vitro. **Methods:** Pixel density scoring of ferroptosis markers: ferroportin, ferritin, NCOA4, cytochrome c, GPX4 and 4HNE, in post-mortem SN of 11 PD cases, 10 PSP/CBD cases and 11 age-matched controls (aged 58-85). A tissue microarray from the same cohorts was immunolabelled with Tyrosine Hydroxylase followed by whole transcriptome sequencing of dopaminergic neurons. Neuronal cultures were challenged with ferroptosis inducers, and inhibitors (Ferrostatin-1, Setanaxib) to explore the pathway. Tau and alpha-synuclein fibrils are being produced to challenge neurons in vitro. **Results:** Positive pixel-density scoring revealed ferritin and ferroportin were significantly changed in dopaminergic neurons with tau/alpha-synuclein, compared to controls, and the ferritin change seemed driven by inclusions. NCOA4, GPX4 and 4HNE increased in tauopathies only. Transcriptomic analysis showed altered ferroptosis-related gene expression in parkinsonism dopaminergic neurons, compared to controls, including downregulated genes for mitochondrial function and protein degradation. Preliminary results indicate Ferrostatin-1 and Setanaxib rescue ferroptosis-induced neuronal death in vitro. **Conclusion:** Ferroptosis is altered in parkinsonisms, possibly driven by inclusions. In vitro preliminary results of ferroptosis inhibition are promising and will be next tested in the presence of fibrils. Our findings highlight ferroptosis pathways as therapeutic targets. **Keywords**: Iron homeostasis – ferroptosis – movement disorders – neurodegeneration – mitochondria

P035

Neuropathological insights into progressive supranuclear palsy: The interplay of combined proteinopathies

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Introduction: Progressive supranuclear palsy (PSP) is a sporadic 4-repeat tauopathy with variable phenotypes, driven by the regional distribution of neuronal loss, tau pathology, and the coexistence of other proteinopathies. **Objectives:** Our aim is to investigate the frequency and severity of copathologies in patients with neuropathological diagnosis of PSP. **Materials & methods:** We analyze a case-series including all PSP cases with neuropathological evaluation in the Brain Bank of the CIEN Foundation in Madrid, Spain. We assessed copathologies according to standardized diagnostic criteria and current staging schemes. This study is nested within the research project 'Progressive Supranuclear Palsy: Identification of Susceptibility Loci, Involved Cell Types, and Molecular Pathways for Drug Development. PSP-DEGESCO Program'. **Results:** Out of the 37 cases included, 24% showed isolated PSP pathology, while the rest exhibited copathologies: argyrophilic grain disease in 46%, Alzheimer's disease neuropathologic change in 38%, and Lewy body pathology in 8% of cases. Corticobasal degeneration overlapped with PSP in 3 cases. One case showed limbic-predominant age-related TDP-43 encephalopathy (LATE) with hippocampal sclerosis, and 2 cases had hippocampal sclerosis with Pick-like hippocampal spherical inclusions without TDP-43 immunoreactivity. One patient had a genetically confirmed diagnosis of Huntington's disease, which coexisted with PSP in the neuropathological examination. **Conclusions:** Comorbid proteinopathies are prevalent in PSP and may have an impact on clinical phenotypes. Although neurodegenerative diseases tend to display typical copathology profiles, atypical cases are detected. Deep neuropathological phenotyping remains crucial for a better understanding of potential disease synergies and their further impact on targeted therapies. **Keywords:** Tauopathy – PSP – copathology

P036

Polyglucosan body disease in a patient with late-onset cerebellar ataxia, down beat nystagmus and tau and TDP43 proteinopathies

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Background: Late-onset vestibulo-cerebellar ataxia (LOCA) is a rare neurological condition. Recently, *FGF14* mutations have been identified as the main cause (75%). We present a LOCA case with down beat nystagmus (DBN) and a likely pathogenic variant in *GBE1*, a gene with mutations usually associated with adult polyglucosan bodies disease (APBD), with cases recently reported to have additional Tau and TDP43 proteinopathies. **Clinical:** Male with episodic and progressive ataxia since his 60s, with instability episodes, DBN, associated cognitive impairment and pyramidal syndrome. Initial genetic and imaging studies did not demonstrate a cause. DBN disappeared spontaneously but had progressive mobility and cognitive deterioration and he died 15 years after ataxia onset. Neuropathological study showed diffuse leukoencephalopathy with cerebellar degeneration, extensive tau astrogliopathy in white matter and limbic TDP43 encephalopathy with hippocampal sclerosis. Abundant polyglucosan bodies were observed in hemispherical and cerebellar white matter and cerebellar cortex, surrounded by tau, phosphorylated TDP43 and neurofilaments. A non-reported homozygotic missense variant c.1300C>G (p.Arg434Gly) was identified in *GBE1* gene. Despite in silico predictors suggesting a deleterious effect, American College of Genetics and Genomics criteria classified it as a variant of uncertain significance. The analysis of *FGF14* gene showed normal expansion. **Conclusion:** APBD may show extensive white matter involvement with broad astrocytic Tau and TDP43 proteinopathies, with an unknown impact in disease progression and clinical manifestations. Despite its known relationship with LOCA, DBN has not been reported in APBD. Genetic and neuropathological studies in patients with LOCA and DBN are relevant to find unexpected underlying neurodegenerative processes. **Keywords**: Polyglucosan disease – corpora amilacea – genomic sequencing – cerebellum degeneration – ataxia

P037

The first Korean case of progressive supranuclear palsy variant with the pallido-nigro-luysian atrophy

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Background: We describe the first case with the pallido-nigro-luysian type Progressive supranuclear palsy (PSP-PNLA). **Clinical histories:** A 70-year-old man visited our movement disorder clinic because of parkinsonian motor symptoms including rest tremors and rigidities on both hands, and bradykinesia. The first brain MRI performed at 70 years old revealed only mild small vessel ischemic change in the corona radiata. FP-CIT PET revealed asymmetrically bilateral decreased FP-CIT uptake in both the putamen and caudate nucleus (CN). Three years later (at age 73), the second b-MRI showed definitely atrophied midbrain compared to the first b-MRI. **Pathological findings:** The whole brain weighed 1,270 grams in the autopsy after he died at age 79. In microscopic examinations, moderate neuronal loss and gliosis were observed in the putamen, subthalamic nucleus (STN), thalamus, and SN. LC and cerebellar dentate nucleus also presented moderate neuronal loss and gliosis. AT8 (phosphorylated tau) immunohistochemistry (IHC) showed moderate neurofibrillary tangles (NFTs) in the entorhinal cortex, nucleus basalis of Meynert (Nbm), SN, and LC. AT8 IHC also showed mild tuft-shaped (or tufted) astrocytes (TAs) in the motor cortex, entorhinal cortex, hippocampus, amygdala, CN, putamen, globus pallidus (GP), thalamus, STN, SN and midbrain tectum. **Conclusions:** The patient showed long disease duration. Like other examples of PSP-PNLA, the neuronal loss and atrophy of the patient focused on the GP, SN and STN. Especially, compared to typical TAs, TAs in PSP-PNLA were apparently smaller in size and were characterized by irregularly shaped, coarse deposits of tau mainly in the perikaryal. PSP-

PNLA has not been reported in Korea. **Keywords:** Progressive supranuclear palsy – Pallido-nigro-luysian atrophy – tufted astrocyte

P038

The role of miR-193b-3p/ PGC-1α pathway in insulin dependent anti-inflammatory response in Parkinson's disease

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Introduction: It has been shown that miRNAs including miR-193b-3p are differentially expressed in Parkinson's disease (PD). miR-193b-3p has the potential to target PPARGC1A, which encodes PGC-1α and is involved in mitochondrial biogenesis, ultimately protecting cells from mitochondrial dysfunction. Furthermore, dysregulation of PGC-1α alters homeostasis in cells and can induce an inflammatory response which is commonly accompanied by metabolic disturbances. **Objectives:** The aim of the present study is to investigate if dysregulation of the miR-193-3p/PGC-1α axis may contribute to the pathological changes observed in the PD brain. **Materials & methods:** Brain tissue was obtained from middle frontal gyrus of non-demented controls and individuals with a PD diagnosis. Using taqMAN-qPCR the expression of miR-193b-3p was determined and in situ hybridization (ISH) and immunohistological analysis were employed to establish the cellular distribution of miR-193b-3p. Functional assays included transfection and knock-down of miR-193b-3p, followed by assaying of gene expression and detection of proteins of the predicted targets. **Results:** We found significantly lower expression of miR-193b-3p in the early stages of PD (PD4) which increased throughout disease progression. Furthermore, the altered expression of PGC-1α suggested a direct inhibitory effect of miR-193b-3p in the brain of individuals with PD. Moreover, we observed changes in expression of insulin after transfection of SH-SY5Y cells with miR-193b-3p, which led to dysregulation in the expression of several pro- or anti - inflammatory genes. **Conclusion:** Our findings indicate that the miR-193b-3p/PGC-1α axis is involved in the regulation of insulin signaling. This regulation is crucial, since insulin induced inflammatory response may serve as a protective mechanism during acute situations but potentially evolve into a pathological process in chronic conditions. This novel regulatory mechanism may represent an interesting therapeutic target with potential benefits for various neurodegenerative diseases. **Keywords:** Insulin – neuro-inflammation – PGC-1α – Parkinson's disease – miRNA - 193b-3p

P039/SY04

Tissue-based studies suggest multiple etiologies contributing to progressive supranuclear palsy

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Introduction: Progressive supranuclear palsy (PSP) is defined by uniform neuropathological features. Cryo-EM studies suggest one predominant type of tau filament. However, there are several clinical phenotypes and progression patterns associated with this pathology and the pathogenesis is unclear. **Objectives:** To perform neuropathology studies on PSP and evaluate variability between cases. **Materials & methods:** We studied PSP cases from the UHN-Neurodegeneration brain disease collection. We performed classical morphological studies, stereology, iron detection methods, antibodies against tau and neurodegenerative disease protein-related epitopes, mitochondrial markers and lysosomal protases, immunogold electron microscopy, transcriptomics methods, seeding assays, and astrocyte culture derived from PSP brains. These were complemented by genetics including HLA genotyping. **Results:** We demonstrate a distinct response of lysosomal, mitochondrial, vascular, and iron pathways and co-pathology molecular signatures compared to other neurodegenerative diseases. These are associated with different inflammatory responses, influenced also by the presence of specific HLA haplotypes. PSP cases show differences compared to other neurodegenerative conditions but also between each other. **Conclusion:** Neuropathology-based studies reveal subgroups of PSP with different HLA haplotypes and neuropathology patterns supporting the notion that the common neuropathology seen in PSP might be associated with various etiopathogenic events, including potential relationships with autoimmune mechanisms. **Keywords:** Etiology – progressive supranuclear palsy – tau

7. Neurodevelopment

P040

Insights into underlying pathophysiology of brain malformations associated with VRK1-related syndrome derived from fetal neuropathology

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Introduction / Background: Biallelic variants in *VRK1* have been described in patients with heterogeneous phenotypes with childhood or adult-onset of progressive upper and lower motor neuron disease. Prenatal onset and neurodevelopmental disorders associated with *VRK1* have rarely been described in the literature. Here, we report the first fetal cases and first neuropathological examination of the brain in patients with pathogenic variants in *VRK1*. **Clinical:** Two fetuses of consecutive pregnancies of second-degree consanguineous parents presented with microcephaly prenatally during the early second trimester. Termination of pregnancy was performed for both cases. Post-mortem examination showed overlapping features including facial dysmorphisms, microencephaly, and brain malformations. These included agenesis of the corpus callosum; absent gyration and reduced neuronal density with a thin cortex, suggestive of a simplified gyral pattern; abnormal corticospinal tracts and fragmentation of the capsula interna; and dysmorphic basal ganglia and hippocampi. Whole exome sequencing identified a homozygous probably pathogenic variant in *VRK1* (NM_003384.3, GRCh38):c.238C>G p.(Leu80Val) in both fetuses. The variant is located in a previously reported cluster close to the ATP-binding site. The parents were heterozygous for the

variant. **Conclusion:** Neuropathological examination in these cases give first insights in the underlying pathophysiological process of biallelic pathogenic variants in *VRK1* in humans. Our findings are evocative of a combination of impaired neuronal proliferation, of a neuronal migration deficit, and abnormal axon guidance. **Keywords**: Fetal neuropathology – simplified gyral pattern – microcephaly – agenesis of corpus callosum – lissencephaly

P041
Multimodal single-cell analyses of the early postnatal Down syndrome brain

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Background: Down syndrome (DS), resulting from genetic perturbations of chromosome 21, is the most common genetic cause of intellectual disability, accounting for approximately one-third of all mild to moderate intellectual disabilities in school-aged children. The cognitive phenotype associated with DS begins in early infancy and progresses to have significant consequences on long-term academic, occupational, and daily functioning. The early onset of these deficits suggests that alterations in neurodevelopmental processes occur during the initial stages of brain development in DS. **Objectives:** This study aims to elucidate the cellular and molecular correlations of cerebral cortical development and maturation in early postnatal DS, as a crucial step towards understanding the biological mechanisms underlying the cognitive and behavioral deficits observed in this condition. **Methods:** Recent advancements in multiomic single-cell and ATAC-sequencing technologies have emerged as powerful tools for investigating brain development in DS. These methodologies enable simultaneous profiling of gene expression and chromatin accessibility, facilitating the correlation between changes in regulatory elements and gene expression patterns. **Results:** Here, we have profiled over 200,000 single cells from the early postnatal dorsolateral prefrontal cortex of postmortem brains affected by Down Syndrome and age-matched control samples. Our results demonstrate a broad homology of cell subtypes between trisomy 21 and control brains and identify non-neuronal cells as the most affected cell type in the early postnatal period. **Conclusion:** Modern "multiomic" sequencing technologies provide unprecedented analysis of the complex gene expression and regulatory perturbations in the developing trisomy 21 brain, offering valuable insights into the underlying mechanisms of DS. **Keywords**: Single-cell sequencing – neurodevelopment – multimodal analyses – ATAC-sequencing – Down syndrome – prefrontal cortex

8. Neuroinflammation

P042
Amyloid beta and tau elicit a differential microglial response in centenarian brains

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Background: Alzheimer's disease (AD), the most common consequence of aging, remains incurable. NFTs and Aβplaques are key neuropathological hallmarks of AD, often accompanied by increased microglial activation, the brain's immune response. Interestingly, similar pathological substrates are found in cognitively healthy centenarians, suggesting these pathologies may not always be detrimental. **Objectives:** To investigate how microglia are activated in healthy aged individuals in relation to AD-like pathology, we compared the presence of microglia activation markers between cognitively healthy centenarians and AD patients. **Methods:** We analyzed brain samples (temporal pole) from 95 cognitively healthy centenarians (median age: 103.5) and 18 AD patients (median age: 67). Immunohistochemical staining was performed for four microglial markers:(1)Iba-1 (pan-microglia),(2)CD11c (phagocytosis/cell adhesion),(3)CD68 (lysosomal phagocytosis), and (4)HLA-DR/DP/DQ (antigen presentation), hyperphosphorylated tau and Aβ. Stained sections were digitized (Olympus VS200) whereafter the percentage of immunopositive cortex was determined(QuPath). Moreover, we determined Thal Aβ phase, Braak NFT stage and CERAD neuritic plaque score. **Results:** In centenarians, CD11c load correlated significantly with Braak NFT stage(r=0.23) and tau load(r=0.35), but not with Thal Aβ phase(r=0.09), Aβ load(r=0.12) or CERAD score(r=0.10). Conversely, CD68 load correlated with Thal Aβ phase(r=0.27), Aβ load(r=0.40), CERAD score(r=0.27) and tau load(r=0.24), but not Braak NFT stage(r=0.09). Iba-1 and HLA-DR did not correlate with tau or Aβ pathology. No associations were observed in the AD cohort. **Conclusion:** In centenarians, we observed microglial CD11c sensitivity to tau while microglial CD68 was sensitive to all AD-associated neuropathological substrates. Together, our results suggest that Aβ and tau elicit a differential microglial response in this centenarian cohort, but not in our AD cohort. **Keywords:** Microglia – centenarians – pathology – amyloid beta – neurofibrillary tangles

P043

Anti-inflammatory and neuroprotective effects of tehranolide and artemisinin in an experimental autoimmune encephalomyelitis model of multiple sclerosis

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Aims: multiple sclerosis (MS) is an autoimmune neurological disease. Artemisinin (ART) is a natural compound derived from extracts of Artemisia annua. ART has anti-inflammatory effects in the experimental autoimmune encephalomyelitis (EAE) animal model, commonly used in MS research. Tehranolide (TEH) is a novel compound similar in structure to ART. We sought to determine whether TEH ameliorates EAE development by targeting proteins and genes involved in this process and comparing its effects to those of ART. **Method:** thirty female C57BL/6 mice were immunized with MOG35–55. 12 days post-immunization, mice were treated with 0.28 mg/kg/day TEH and 2.8 mg/kg/day ART for 18 consecutive days. We assessed proinflammatory and anti-inflammatory cytokines levels in serum and splenocytes by ELISA and the mRNA expression level of T cell differentiation and myelination genes by qRT-PCR. **Results:** Administration of TEH and ART markedly alleviated EAE symptoms. The TEH-treated group showed a significant decrease in IL-6, IL-17, and IL-1;

however, ART had less significant effects. Moreover, TGF-β, IL-4, and IL-10 genes were stimulated by ART and TEH in the spinal cord, while the treatments did not affect IFN-γ expression. Both treatments dramatically increased the expression of FOXP3, GATA3, MBP, and AXL. The compounds exerted no changes in RORγt, nestin, Gas6, Tyro3, and Mertk mRNA expression levels in the spinal cord. **Conclusions**: we discovered that both TEH and ART can effectively modulate the inflammation and myelination genes in EAE. TEH demonstrated a higher potency than ART, making it a possible future candidate for MS therapy. **Keywords**: Myelin – inflammation – natural product – multiple sclerosis

P044

Characterizing the inflammatory cell infiltrate and neuropathological features in cerebral amyloid angiopathy-related inflammation

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Introduction: Spontaneous cerebral amyloid angiopathy-related inflammation (CAA-ri) occurs in a rare subset of cerebral amyloid angiopathy (CAA) and has been suggested as a model for Amyloid-Related Imaging Abnormalities (ARIA) reported in Alzheimer's patients immunized against amyloid-beta (Aβ). **Objectives:** Neurosurgical biopsies of CAA-ri present an opportunity to understand the immune response associated with ARIA. **Materials & methods:** Neurosurgical biopsies from 19 CAA-ri and 44 CAA cases underwent neuropathological diagnosis/ analysis, immunolabelling for T-lymphocytes (CD4, CD8), and microglia/ macrophage (CD68) followed by quantification. **Results:** CAA-ri demonstrated more frequent extension of vascular Aβ into the surrounding parenchyma and capillary angiopathy compared to CAA ($p=0.016$ and $p=0.025$). Features of vasculopathy and plaque removal were more frequent in CAA-ri than CAA ($p<0.001$ and $p<0.001$). CAA-ri had increased perivascular CD8+, CD4+ and CD68+ cells vs. CAA ($p<0.001$, $p=0.036$ and $p=0.0372$). The proportion of vessels with, and severity of, intramural CD8+ T-lymphocytes was greater in CAA-ri vs. CAA ($p=0.001$ and $p<0.001$); while intramural CD4+ and CD68+ staining were non-significant. In CAA-ri, a higher proportion of vessels had intramural and perivascular CD8+ vs. CD4+ T-lymphocytes ($p=0.001$ and $p<0.001$) and more CD8+ T-lymphocytes per perivascular area were observed vs CD4+ T-lymphocytes ($p=0.041$). **Conclusion:** The inflammatory component of CAA-ri comprised lymphocytes, more commonly CD8+, and macrophages/microglia including multinucleate forms. In CAA-ri, intramural inflammation was frequent but not ubiquitous. CAA-ri was associated with more severe vascular Aβ, capillary angiopathy, vascular damage and features of plaque removal. Analysis of APOE4 phenotype and correlates with clinical/ radiological outcomes is ongoing. **Keywords**: Cerebral amyloid angiopathy-related inflammation – amyloid-beta – immunotherapy – neuroinflammation – neuropathology – Alzheimer's disease

P045

Combined morphology and protein profiling grades microglial reactivity across neurodegenerative proteinopathies and brain regions

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Introduction: Transcriptomic studies have highlighted significant diversity of microglia and their reactivity to misfolded proteins across different neurodegenerative diseases and brain regions. **Objectives:** Investigate such microglial differences on protein and morphology level when comparing Alzheimer's disease (AD) and Creutzfeldt-Jacob's disease (CJD) using novel targeted proteomics together with digital morphological profiling. **Methods:** Post-mortem human FFPE brain tissue from both the frontal and occipital neocortex of AD (n=5), CJD (n=5) and control (n=2) patients were processed in Nanostring's GeoMx Digital Spatial Profiler platform to obtain immunofluorescence images of IBA1-stained cells. Targeted antibody-based proteomics was performed on isolated IBA1+ segments in the platform, while digital morphological profiling was conducted on segmented IBA1+ objects through CellProfiler. Combined quantitative reactivity grading was established using both protein and morphology data with trajectory analysis in R. **Results:** Morphological profiling of IBA1+ objects distinguished a ramified subtype and two ameboid subtypes. The ramified subtype was the most common subtype in controls and was associated with homeostatic microglia proteins like TMEM119 and P2ry12. One of the ameboid subtypes was linked to inflammatory markers, like HLA-DR and CD11c, and was more prevalent in CJD patients. Reactivity grading could order the patient groups, with lowest scores for controls and highest scores for CJD. Most interestingly, occipital cortex samples within both AD and CJD patients had higher reactivity scores than those from the frontal cortex. **Conclusion:** Both protein expression and morphometrics of IBA1+ cells can be synergistically joined to grade the reactivity of microglia across different proteinopathies and brain regions. **Keywords:** Microglia – prion disease – Alzheimer's disease – morphology – proteomics – spatial biology

P046/SY11

Detailed characterization, evolution and early events of brainstem tau pathology in anti-IgLON5 disease

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Introduction: Anti-IgLON5 disease is a newly identified rare form of autoimmune disorder characterized by anti-IgLON5 antibodies that, via an as-yet-unidentified mechanism, are associated with a brainstem-dominant tau pathology. Recent research indicates that tau pathology may develop in a time-dependent manner. This study aimed to precisely characterize the different tau phosphorylation steps and establish a sequence of markers at the different severity stages in an autopsy cohort. **Methods:** We used immunohistochemistry to analyze the medullary region of 14 autopsy cases of anti-IgLON5 disease with different severity grades of tau pathology and varying disease durations, from 6 to 180 months. We used 13 antibodies for different tau phosphorylation sites. The presence and severity of tau pathology were assessed semi-quantitatively and compared to one PSP case and two neurologically healthy controls. As a proof of concept, we also performed a cell culture with rat hippocampal neurons and antibody incubation. **Results:** We identified different tau phosphorylation sites in anti-IgLON5 disease and were able to determine a sequence of phosphorylation steps based on the disease duration. The development of cytoplasmic tau pathology seems to occur only in later disease stages, whereas alterations of the nuclear envelope were evident in the early stages. **Conclusion:** Different tau phosphorylation steps occur during the progression of anti-IgLON5 disease. The establishment of a sequence of pathological disease markers adds support to the hypothesis that tauopathy might indeed be a time-dependent phenomenon. Furthermore, it may aid in the neuropathological identification of cases with mild pathology, and provides insight into the earliest stages of tauopathy. **Keywords:** Anti-IgLON5 disease – antibodies – neuroinflammation – tauopathy

P048/SY05

In-depth study of viral spread and immune cell signatures in human BoDV11 encephalitis

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Introduction: Borna disease virus 1 (BoDV-1) infection is a recently identified cause of fatal encephalitis in humans, with limited knowledge about its pathogenesis. **Objectives:** This study investigates the CNS

distribution of BoDV-1, focusing on regions with varying viral loads, and examines the associated immunological responses to better understand Bornavirus encephalitis (BVE). **Methods:** Complete coronary and sagittal brain sections from three individuals who died of BVE were embedded and stained immunohistochemically for the BoDV-1 nucleoprotein. The stained sections were digitally reconstructed, and the number of BoDV-1-positive cells was quantified using QuPath software, generating the semiquantitative H-Score, which is based on staining intensity and proportion of stained cells. Furthermore, viral loads in each tissue block were quantified by qPCR. Additionally, immunological profiling was performed using Nanostring's nCounter technology, a transcriptome-based method, to measure approximately 700 genes involved in neuroimmunological processes. **Results:** The basal ganglia, brainstem, and thalamus are severely affected, as determined by both immunohistochemistry and qPCR, while the cerebellum typically exhibits relatively low viral intensities. The concentration of viral loads in the basal ganglia, brainstem, and thalamus may provide insights into mechanisms of viral entry and brain propagation. The immunological profiles revealed that CD56dim natural killer cells exhibited the highest expression in regions with high viral loads compared to low viral loads. **Conclusion:** This research marks a significant advancement by providing the first in-depth analysis of CNS viral load distribution and immune profiles in Borna virus encephalitis. As a model for neurotropic viral infections, understanding BVE's pathogenesis and mechanisms is essential. **Keywords:** Borna disease virus 1 – encephalitis – immunology – viral distribution – pathogenesis

P049

Mapping activated white matter microglia associated with subpial cortical grey matter lesions in MS.

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Introduction: Subpial grey matter lesions are a pathological feature in multiple sclerosis (MS) linked to severity but rarely captured by routine neuroimaging. **Objectives:** To identify if human leucocyte antigen-D (HLA) + microglia/macrophages represent a tissue-based marker of subpial grey matter lesions. **Patients and methods:** Immunostained and digitized slides of HLA and myelin oligodendrocyte glycoprotein (MOG) from the superior frontal gyrus (SFG; n = 40), cingulate gyrus (CG; n = 42), and primary visual cortex (PVC; n = 52) were analyzed from 59 cases of progressive MS (34 female, median age = 60, IQR 20; REC18/WA0238). Quantification of HLA percentage area throughout the white matter (WM) was radially mapped based on distance away from the subpial grey matter lesion core. **Results:** Subpial grey matter lesions were identified from 67 donor blocks. Initial analysis of the %HLA in white matter underlying lesion grey matter (NWM-L) showed a robust increase in CG (1.80%, p=0.03), PVC (1.94%, p=0.008) and combined (1.67%, p=0.0003). Increased HLA signal in NWM-L was also associated with %cortical grey matter demyelination (r=0.210, p=1.8x10-10). **Conclusion:** We mapped %HLA to the entire WM with a radial map to demonstrate its association with subpial grey matter lesions. Activated WM microglia could represent a novel neuroimaging marker of subpial grey matter lesions. **Keywords:** Lesion – subpial – microglia – HLA – mapping – QuPath

P050

Metagenomic sequencing in neuropathology diagnostics

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Introduction: Central nervous system (CNS) infections are severe and potentially life-threatening. Rapid identification of the causative pathogen is essential for initiating targeted and effective therapy, thereby improving patient outcomes. **Objectives:** This study evaluates the utility of metagenomic sequencing in routine diagnostics for detecting CNS pathogens from cerebrospinal fluid (CSF) and formalin-fixed paraffin-embedded (FFPE) samples. **Materials & methods:** Between 2023 and 2024, 99 patients with clinical and/or histopathological suspicion of CNS infections were enrolled. DNA metagenomic sequencing was performed on 95 samples, RNA sequencing on 7 samples (6 of which also underwent DNA sequencing), and total nucleic acid (tNA) sequencing on 3 samples, using an Illumina NextSeq platform. Sequencing data were analyzed with the IDseq pipeline and compared against control samples. **Results:** The cohort included 36 females and 63 males with a median age of 45 years. Sequencing was performed with a median read depth of 35 million reads (IQR: 28–51 million reads). Pathogens were identified in 38 patients (38%). Bacterial infections predominated (25/38, 66%), with 5 cases showing polybacterial infections. Notably, three polybacterial CNS abscesses suggested an oral origin of the bacteria. Fungal, viral, and parasitic pathogens were detected in 5, 4, and 4 cases, respectively. Co-infections were notable in two immunosuppressed patients: one with bacterial and cytomegalovirus co-infection and another with fungal and cytomegalovirus co-infection. **Conclusion:** Metagenomic sequencing is a valuable diagnostic tool for identifying clinically relevant pathogens in FFPE and CSF specimens, enabling the detection of both mono- and mixed CNS infections.
Keywords: Metagenomics – molecular diagnostics – next-generation sequencing – CNS infections

P051/SY05

Multiplexed viral detection in brain tissue – methodological challenges and possibilities

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Introduction: Following initial claims for viral detection in CNS tissue during the SARS-CoV-2 pandemic, subsequent studies revealed reduced specificity of immunohistological stains and common misidentification of cellular structures as viral in studies using electron microscopy. The resulting scientific debate on viral presence in the CNS highlights the utmost importance of robust methods for specific detection of viral products. **Objectives:** Therefore, we want to use an autopsy collection of

multiple centers to investigate the challenges of SARS-CoV-2 detection in the brain on RNA and protein level. **Patients & methods:** We generated tissue microarrays containing 380 thalamic and cerebellar samples from donors deceased of COVID-19 (n = 134) and age-matched prepandemic controls (n = 71). SARS-CoV-2 transcriptional abundance and interferon-related gene expression were analyzed using 10x Xenium probe-based spatial transcriptomics, with pulmonary tissue of COVID-19 donors serving as control for active infection and three HSV1 probes serving as control for neurotropism. For orthogonal validation, digital PCR and immunohistochemistry were performed on a subset of samples. **Results:** Pulmonary controls contained SARS-CoV-2 RNA counts and protein, and brain samples from one systemically HSV1-infected but not previously diagnosed donor showed cells positive for all three HSV1 probes and protein staining. Among all other samples, significant but weak signal for both SARS-CoV-2 and HSV1 probes was restricted to granule cell areas of few PCR-negative cerebellar samples and correlated closely with negative control background. Interferon-related gene expression in COVID-19 brain samples was elevated in comparison to controls, but lower than in pulmonary tissue. **Conclusion:** Taken together, these results point to a bystander activation of the CNS during systemic COVID-19 infection, rather than true neurotropism of SARS-CoV-2 and highlights the challenges of methodological cross-validation. **Keywords:** SARS-CoV-2 – probe-based spatial sequencing – multi-center – validation – interferon

P052/SY11
Neuropathology of GABA-BR auto-immune encephalitis

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Introduction: Autoimmune encephalitis (AE) with antibodies against the extracellular GABA-B1 (GABABR) receptors is clinically characterized by rapidly progressive dementia, seizures, and severe neuropsychiatric symptoms. The GABA-BR mediates inhibitory signals, suppresses high activity states in neuronal networks and is mainly expressed in hippocampus, amygdala, thalamus and cerebellum. Patients with anti-GABABR encephalitis usually strongly improve after treatment with immunotherapy. Previously, it has been shown in anti-NMDAR receptor (NMDAR) that B cells play an essential role in the pathogenesis of AE with extracellular antibodies. However, the immunopathology of GABA-B encephalitis in human brain has not been systematically described. **Objectives** Analyze GABAB encephalitis immunopathology in human brain tissue and compare with anti-Hu encephalitis and non-neurological control brains. **Methods** In post-mortem brain tissue from 4 GABA-BR encephalitis cases, 2 anti-HU encephalitis cases and 4 controls we performed immunohistochemistry for microglia (CD68, HLA-DR), T cells (CD3, CD4, CD8, GranB), B cells (CD20, CD79a), plasma cells and antibodies (CD138, IgG), synapses and axons (synaptophysin, APP, neurofilament) in 6-9 brain regions per donor. **Results** In GABA-BR encephalitis we find a limbic encephalitis predominantly affecting hippocampus and amygdala with perivascular cuffing of lymphocytes. There is a prominent CD8+ T cell response infiltrating the brain parenchyma and perivascular space with granzyme B positivity. B cells were mostly located perivascular. **Conclusion** Anti-GABA-BR encephalitis neuropathology is characterized by limbic encephalitis with a prominent and active cytotoxic T cell response, in addition to B cells. We show that T cell pathology plays an essential role in this subtype of AE with extracellular antibodies. **Keywords**: Dementia – neuropathology – autoimmunity – neuroinflammation – T cells – B cells

P053

Pegivirus encephalitis: A distinctive viral CNS infection in immunosuppressed patients

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Introduction: Pegivirus hominis (HPgV-1), a single-stranded RNA flavivirus, has been considered non-pathogenic in humans. We report a series of four chronically immunosuppressed patients with encephalomyelitis associated with HPgV-1 infection. Our focus is on tissue-based characterization of the virus-host response using spatial transcriptomics. **Objective:** Investigate the association between HPgV-1 infection and encephalomyelitis in immunosuppressed patients. **Materials & methods:** Clinical and radiological data were evaluated, and HPgV-1 RNA was quantified in CSF, serum, and autopsy samples using RT-qPCR. Spatial transcriptomics was performed on brain autopsy samples using the Xenium In Situ Platform (10x Genomics), targeting 300 genes. Results: MRI scans revealed hyperintensities on T2/DWO-weighted images along the pyramidal tract. Post-mortem tissue analysis showed higher HPgV-1 RNA viral loads in CNS samples compared to non-CNS tissues. The genetically distinct HPgV-1 population in the CNS suggests independent viral replication in the CNS. Histologically, lesions showed infiltration of CD68+ macrophages and CD8+ T cells, myelin and axonal destruction, and upregulation of HLA-DR. Using Xenium, we examined the spatial heterogeneity of the viral host response, such as the spatial distribution of interferon-related genes. We correlated this with histopathology using image alignment tools. **Conclusion:** Symmetrical bilateral involvement of the pyramidal tracts should prompt testing for HPgV-1 RNA. The distinct HPgV-1 population in the CNS indicates independent replication in the CNS. It remains unclear which host or viral factors determine potential neurotropism leading to encephalitis, as HPgV-1 RNA is present in 5% of the population.
Keywords: Virus – spatial transcriptomics – encephalomyelitis

P054

Phenotypic alterations in astrocytes in fungal infections of central nervous system

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Introduction: Astrocytic response in CNS infections has not been systematically evaluated. Fungal infections, the incidence of which is rising due to increasing immunosuppressive states, ecological changes etc. **Patients and methods:** Clinical, demographic and neuropathological changes of twenty-nine patients with CNS fungal infections were reviewed. Immunohistochemistry for GFAP and AQP4 performed to evaluate astrocytic response and BBB alterations. **Results:** Twenty-nine cases including mucormycosis-11, invasive aspergillosis-2, aspergilloma-7, cryptococcoma-2, cryptococcal meningitis-2 and chromoblastomycosis-4, and candida-1 infection were studied. Granulomatous response was prominent in aspergilloma and cryptococcoma. Astrocytes showed varied morphologies dictated by histological tissue reaction patterns. Granulomatous inflammation and abscess showed loss of astrocytes and AQP4 within necrotic centers, dysmorphic astrocytes with fragmented and beaded processes with perivascular accentuation of AQP4 in periphery of lesion and hypertrophic astrocytes in adjoining parenchyma with diffuse AQP4. Stellate astrocytosis and increased perivascular AQP4 were seen in cryptococcal meningitis. Cases with diffuse cerebritis showed stellate astrocytes with marked loss of AQP4 expression. **Conclusions:** Varied tissue response of CNS fungal infections were associated with distinct astrocytic phenotypes. Dysmorphic changes can impact astrocytic function leading to altered BBB with subsequent edema and neuronal dysfunction causing morbidity. Understanding glial pathophysiological changes may help abrogate neurological sequelae in survivors.
Keywords: Astrocytes – fragmentation – BBB – fungal infection – CNS

P055

Viral meningoencephalitis: Evaluation of the relationship between forensic histopathology and microbiology

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Introduction: Viral meningoencephalitis, an inflammation of the central nervous system, affects both the parenchyma and meninges. Common causative agents include Human Herpes Virus 6-7, Herpes Simplex Virus, Epstein-Barr Virus, Cytomegalovirus, Parvovirus, Enterovirus, Adenovirus, Varicella-Zoster Virus, and Paramyxovirus. Histopathological findings include parenchymal damage, reactive astroglial and microglial proliferation, microglial nodules, neuronophagia, inflammatory infiltration (perivascular or widespread), and vascular changes. **Objectives:** To examine the relationship between viral agents in meningoencephalitis and autopsy-based histopathological findings. **Materials & methods:** Between 2018–2023, hematoxylin and eosin-stained slides of brain, cerebellum, brainstem, and meninges from 45 PCR-tested cases of suspected viral meningoencephalitis were re-evaluated. Identified viral agents were documented and correlated with histopathological findings. **Results:** Among 45 cases (29 male, 16 female; mean age: 22.4 years), inflammation was present in the brain, cerebellum, and brainstem in 19 cases; cerebellum and brainstem in 4; brain alone in 17; cerebellum alone in 2; and brainstem alone in 3. Perivascular inflammation was observed in 35 cases, intraparenchymal in 2, and both patterns in 8. In 42 cases, inflammation was mononuclear, while 3

showed a mixed pattern. PCR detected parvovirus B19 (6), varicella-zoster (2), herpes simplex-1 (1), cytomegalovirus (1), and adenovirus (1). All PCR-positive cases exhibited inflammation in all three regions. The CMV-positive case showed mixed inflammation, whereas the others exhibited mononuclear infiltration. **Conclusion:** Mononuclear perivascular inflammation in autopsies with undetermined causes of death warrants further investigation for viral meningoencephalitis, integrating histopathological and microbiological analyses for diagnosis. **Keywords:** Meningoencephalitis – autopsy – virus

P148

Rare 'subcortical ribbon sign' in childhood primary angiitis of the central nervous system

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Objectives: Childhood primary angiitis of the Central Nervous System (cPACNS) is a rare autoimmune inflammatory disease. Approximately 5% of PACNS are characterized by isolated tumor-like lesions. 'Subcortical ribbon sign' in cPACNS was rare. **Patients & Methods:** The patient was a 9-year-old boy. After a fever, he presented central facial paralysis and hypersomnia who was diagnosed by "viral encephalitis". Cranial CT showed hyperdense lesions in the left basal ganglia area (Figure 1). The magnetic resonance imaging (MRI) showed the left side paraventricular, basal ganglia area, thalamus, cerebral peduncle and right basal ganglia T2WI hyperintensities and the lesions were enhanced on the enhanced MRI (Figure 2). The subcortical ribbon sign, a neuroimaging feature observed on diffusion-weighted imaging (DWI) or T2-weighted fluid-attenuated inversion recovery (Flair) sequences. Cho/NAA=6.8 on MRS (Figure 3). During the period, he had a persistent fever, the maximum temperature was 39.9℃, and then the right limbs were weak. Next-generation sequencing (NGS), the antibodies (APQ4, MOG, GEAP, MBP, NMDAR, AMPAR1, AMPAR2, LGI1, CASPR2, and GABPABR), oligoclonal bands were all negative. A biopsy of the 'left lateral ventricle side' was performed. **Results:** Telangiectasia with hemorrhage and perivascular infiltrates were observed. (Figure 4). **Conclusion:** cPACNS was diagnosed based on pathology. Subcortical ribbon sign on MRI mimicked Adrenoleukodystrophy dystrophy or Neuronal intranuclear inclusion disease. The Cho/NAA=6.8 was easily misdiagnosed as a tumor, which was because of rapid myelin disintegration. Subcortical ribbon sign demonstrated progression on vascular imaging, without apparent clinical or angiographic predictors.

Keywords: Childhood primary – angiitis of the central nervous system – subcortical ribbon sign

9. Neuromuscular disorders

P056

ERN EURO-NMD AI Pathology Hub: A first-line automated analysis to improve accessibility and reduce costs of muscle biopsy analysis in Europe

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Introduction: Myopathies encompass a broad spectrum of both inherited and acquired neuromuscular disorders. Interpreting muscle biopsies requires the analysis of a wide and expensive battery of histochemical and immunohistochemical techniques by experienced neuropathologists. Next-generation sequencing and serological diagnosis have changed the indication for muscle biopsies, limiting both access and interpretation skills in Europe. Nevertheless, they remain indispensable to identify and characterize inflammation, to validate pathogenicity when variants of uncertain significance are identified on genetic testing, or when novel genes are discovered. **Objectives:** 1) To implement a first-line machine-based interpretation of digital images from HE-stained muscle biopsy sections to predict dystrophic, inflammatory, neurogenic, and myopathic patterns, for appropriate selection of second-line immunohistochemical techniques. 2) To expand access to muscle biopsy analysis across Europe and reduce associated costs. **Methods:** For the first machine learning phase, Hematoxylin-Eosin (HE) stained scanned sections from 89 muscle biopsies including 30 muscular dystrophies, 50 inflammatory myopathies, 9 neurogenic disorders, and 40 histologically healthy controls were analysed with MyoSOTHES, an automated myofiber segmentation tool, combining QuPath and Cellpose. **Results:** MyoSOTHES detected: A) A higher fiber size variability distribution in all patterns compared to controls. B) Significantly higher nuclear internalizations in dystrophic, inflammatory, and neurogenic patterns. C) Inflammatory infiltrates, identified as areas of increased nuclear density, were significantly higher in inflammatory myopathies compared to controls. **Conclusions:** This preliminary analysis confirmed that MyoSOTHES is an efficient tool to recognize three out of the four selected myopathological patterns. Further analysis on a larger cohort of biopsies, including the myopathic pattern, is ongoing to complete the machine learning phase. Predicting myopathological patterns using digitally scanned HE sections from blinded muscle biopsies will be the next step to implement a first-line machine-based interpretation. **Keywords:** Muscle biopsy – digital pathology – artificial intelligence – MyoSOTHES – myopathology

P057

FHL1-related myopathy with myofibrillar pathology and reducing bodies

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Introduction: Myofibrillar myopathies comprise a clinical and genetic heterogeneous group of inherited muscle disorders with unifying histopathological features. **Objectives:** To describe a family with myofibrillar myopathy focusing on pathological clues to the genetic diagnosis. **Patients & methods:** Retrospective review of clinical, neurophysiological, histopathological and genetic data from four related patients with FHL1-related myofibrillar myopathy. **Results:** All affected males (3 brothers and 1 cousin) presented with adult-onset lower limb weakness (mean age of onset 50 years), muscle cramps and elevated CK levels (500-1000 U/L). Neurologic examination revealed progressive mild to moderate limb girdle weakness in all patients and scapular winging in one, with no contractures. EMG studies showed a predominantly myopathic or mixed pattern (with neurogenic features). Cardiac workup was normal. Two moderately affected patients performed deltoid muscle biopsies disclosing severe myopathic changes with similar myofibrillar pathology, rimmed vacuoles, few cytoplasmic bodies and some structures partially highlighted with menadione-NBT reaction, compatible with reducing bodies. Another mildly affected patient showed only a slightly myopathic pattern with no internal structure abnormalities. Genetic testing found a new missense hemizygous variant (c.407T>G; p.Ile136Ser) in FHL1 gene in all affected patients, predicted to be pathogenic in bioinformatics analysis. The familial segregation pattern also supported its pathogenicity. **Conclusion:** Among myofibrillar myopathies, the presence of reducing bodies and a familial X-linked pattern of transmission may constitute important clues to suspect a FHL1-related myopathy. Although highly specific, the presence of reducing bodies showed intrafamilial heterogeneity and variable staining intensities with menadione-NBT. **Keywords:** Muscle biopsy – myofibrillar myopathy – reducing bodies – menadione-NBT – FHL1

P058

Inter-institute agreement for the determination of the intraepidermal nerve fiber density

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Introduction: Diagnosis of small fiber neuropathy relies in part on quantification of intraepidermal nerve fiber density (IENFD) in skin punch biopsies. IENFD is an in-house in vitro diagnostic tool for which external quality control is required, but for which there is no established round robin test. **Objectives:** Here we describe the first analysis of the inter-institute agreement of the in-house IVD IENFD between the Institutes of Neuropathology in Vienna (MUV) and Zurich (USZ). The study represents a pilot project to seek an inter-institutional comparison between several institutes. **Materials & methods:** Punched skin biopsies were obtained from 6 autopsies (approved by the KEK Zurich), from which each institute received a sample from the same position and performed in-house

analysis for IENFD. The IENFD was determined by three different observers per institute. The inter-rater agreement was then estimated by the interclass correlation coefficient (ICC), and the inter-institutional agreement was analyzed by Bland-Altman blot. **Results:** The inter-rater agreement at USZ and MUV was determined to be "excellent" and "good", respectively. The analysis of the differences in mean nerve fiber density per sample between the two institutions shows that the IENFD values were slightly higher at the USZ than at the MUV. **Conclusion:** We have, for the first time, successfully used skin punch samples from autopsies to determine both the intra-observer variance and the inter-institute agreement in determining the IENFD. The initial data collected form the basis for planning an inter-institutional comparison with more institutes as a next step. **Keywords**: Peripheral neuropathy – small fiber neuropathy – Intraepidermal nerve fiber density – in-house in vitro diagnostics – quality management – external quality control

P059/SY02

Reclassifying muscular involvement in systemic sclerosis: Unveiling unique histopathological signatures and overlaps with immune myopathies features

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Introduction: Skeletal muscle involvement is common in systemic sclerosis (SSc), but its classification and clinical significance remain unclear. We performed detailed histological, immunofluorescent, and transcriptomic characterization of SSc myopathy (SScM) to define underlying disease mechanisms. **Materials & methods:** Deltoid biopsies from 83 SScM patients categorized as fibrosing, inflammatory, or necrotizing by European Neuromuscular Centre criteria, along with 11 controls, underwent quantitative morphometry of fibrosis, fiber atrophy, and microangiopathy. Transcriptomes of 32 samples were analyzed by RNA sequencing. Extensive clinical data were collected on 62 patients. **Results:** All SScM subgroups showed increased endomysial fibrosis, type II fiber atrophy, capillary loss, and vessel enlargement versus controls. Transcriptomics revealed overexpression of inflammatory, interferon, fibrotic, and microvascular gene sets, most pronounced in inflammatory/necrotizing subgroups. Despite histological heterogeneity, SScM exhibited shared pathological characteristics distinct from controls. **Conclusion:** SScM manifests as a spectrum of muscle pathology unified by core microangiopathic, fibrotic, and transcriptional alterations. Usual subclassification obscures these commonalities. Defining SScM by quantifiable histological and molecular hallmarks could better guide diagnosis and therapy targeting shared dysregulated processes underlying muscle damage across the disease spectrum. **Keywords:** Systemic sclerosis – immune myopathy – myositis – muscle biopsy

P060

Role of the chaperonin TRiC component CCT3 in neurodevelopment

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Introduction: We have recently contributed to the discovery of a group of neurodevelopmental diseases caused by mutations in components of the chaperonin tailless complex polypeptide 1 ring complex (TRiC), called TRiCopathies. TRiC is composed of subunits *CCT1-8* and acts as a chaperone that is required for folding of 10% of proteome, including actin and tubulin. Brain MRI scans of patients with *CCT* mutations, especially in *CCT3* showed reduced white matter. **Objectives:** Here, we aimed at better understanding the pathophysiological role of cct3 in neurodevelopment using zebrafish as a model system. **Methods:** We have generated two CRISPR/Cas9 loss-of function alleles of the orthologous zebrafish *cct3* gene. Combining these mutant alleles with transgenic zebrafish lines allows us to image different cell types and subcellular structures in the nervous system on a spinning disc confocal microscope. Furthermore, we performed electron microscopy. **Results:** We could show that *cct3* is essential for the biology of the nervous system. In particular, we demonstrated that *cct3* mutant zebrafish do not form normal myelin sheaths and that this is associated with early death of neural crest-derived cells, which were particularly vulnerable to loss of *cct3* function. Furthermore, we observed profound abnormalities of actin and microtubules as well as severely disturbed axonal transport of organelles in peripheral motor axons. **Conclusion:** Our results underscore the conserved role of cct3/TRiC in the developing nervous system. The essential role in myelination as well as in axonal transport explains at least in part the pathophysiological mechanisms in TRiCopathies in humans. **Keywords**: TRiCopathies – cct3 – myelination – axonal transport

P061/WS13

Unravelling molecular mechanisms in late-onset Pompe's disease: Insights from proteomic analyses

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Introduction: In Pompe disease, mutations in GAA lead to a vacuolar myopathy with glycogen accumulation and increased autophagy. However, the underlying pathology is not fully understood. Molecular studies of human skeletal muscle are rare. **Objectives:** To better understand disease mechanism, we performed a molecular analysis of muscle biopsies from patients with late onset Pompe disease (LOPD) and age-matched controls. Results were verified by immunofluorescence studies. **Materials & methods:** Skeletal muscle samples with a varying degree of pathology, as assessed by standardized histological grading were analyzed. Mass spectrometry (MS) was performed on muscle biopsies from 21 ERT naïve LOPD patients (mean age 47.3 years, 43% female) and 11 controls (mean

age 43.4 years, 50% female). **Results:** MS analysis identified a total of 830 proteins, with significant deregulation of 68 protein (DEPs) in LOPD (44 downregulated and 24 upregulated) compared to controls. Among others, sarcomere proteins were downregulated in LOPD. Correlating MS data with muscle pathology revealed a significant positive association for Vesicle-Associated Membrane Protein B/C (VAPB), involved in the endoplasmic reticulum unfolded protein response and RPS9, which plays a role in ribosome biogenesis, DNA repair, and developmental regulation, with increased muscle pathology. **Conclusion:** This study highlights that a variety of regulatory processes contribute to impaired skeletal muscle function in Pompe disease. The deregulation of proteins associated with sarcomeric, and ribosomal function may aggravate the disease progression in striated muscle fibers. The results also suggest that increasing muscle pathology is associated with different molecular processes during disease progression. **Keywords**: Myopathy – glycogen storage disease – vacuolar myopathy – proteomic

P062/SY07

Von Willebrand Factor (vWF) as a biomarker of acute nerve damage in vasculitic neuropathy: Evidence from a combined analysis of serum proteome and sural nerve pathology

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Introduction: Neuropathy is a common, painful and disabling manifestation of vasculitis. It is diagnosed via nerve biopsy, but its diagnostic yield varies. **Objectives:** To identify novel blood-based biomarkers that improve diagnostic accuracy in patients with vasculitic neuropathy. **Patients & methods:** We retrospectively assembled a cohort of 35 patients who underwent nerve biopsy, with corresponding blood samples available for each patient. 10 patients had a clinical diagnosis of vasculitis. Additionally, we collected control blood samples from 12 patients with psychosomatic disorders. We applied an unbiased mass spectrometry approach to identify protein signatures and correlated protein abundance with histopathological parameters. We quantified the extent and acuity of nerve damage in a combination of manual and automated image analyses of immunohistochemically stained sections, semithin sections and teased fiber preparations. We considered a correlation coefficient of >0.5 as clinically meaningful and focused on proteins with at least a 2-fold up/downregulation. **Results:** We identified 15 serum proteins with a 2-fold up/downregulation compared to controls. Out of those, none showed a correlation with the extent of nerve damage detected in nerve biopsies. One protein (vWF) demonstrated a positive correlation with the acuity of nerve damage (n=27; r=0.55; p=0.003; Spearman rank analysis). VWF-levels in patients with a clinical diagnosis of vasculitis were significantly elevated compared to controls (9-fold upregulation; n vasculitis=7 n controls=12; p=0.02). **Conclusion:** vWF is released in response to endothelial damage and discussed as a marker for disease activity in patients with vasculitis. Our

analysis identifies vWF as a biomarker of acute nerve damage in patients with vasculitic neuropathy. **Keywords:** Vasculitic neuropathy – biomarker – vasculitis – nerve biopsy – histopathology – proteomics

10. Neurovascular pathology

P142/WS05

Study of cerebral amyloid angiopathy severity and distribution according to the presence of intracerebral hemorrhage and/or Alzheimer's disease neuropathological change

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Introduction: Cerebral amyloid angiopathy (CAA) is characterized by Amyloid-β (Aβ) deposition in cerebral blood vessels. It is associated with aging and highly prevalent in Alzheimer's disease (AD). CAA is the leading cause of lobar intracerebral hemorrhages (ICH) in the elderly, although the underlying mechanisms driving hemorrhaged in CAA remain unknown. **Objectives:** To identify factors linked to ICH in pathologically confirmed CAA and evaluate CAA severity and distribution based on ICH presence and AD pathology. **Methods:** We studied 205 brains with neuropathologically confirmed CAA (median age: 85y, 67.3% women). Samples from frontal and occipital lobes, hippocampus, and cerebellum were analyzed. Aβ-immunostaining and the Vonsattel scale (Grades G0–G4) were used to evaluate CAA severity. **Results:** ICH occurrence was not associated with age, sex or capillary Aβ. CAA severity was the strongest factor linked to ICH ($p<0.001$). Patients with ICH exhibited higher G3+4 Vonsattel grades (53%), evidencing vascular remodeling and fibrinoid necrosis, compared with patients without ICH or AD pathology (12.5%, $p<0.001$). However, patients presenting AD pathology without ICH also showed higher CAA grades (34.1%), whereas patients with ICH and AD pathology showed a cumulative effect, harboring the highest percentage of patients with G3+4 (84.7%). Finally, patients with ICH without AD pathology (35%) and patients with AD without ICH (34,1%) showed a similar grade distribution ($p=0.3322$). **Conclusions:** We confirmed that vascular secondary changes are associated with ICH in severe CAA, although these features were also present in AD without symptomatic ICH, suggesting that additional triggers may drive ICH in CAA. **Keywords**: CAA – Alzheimer hemorrhage – amyloid – angiopathy

P072/WS05

Topical hemostatic agents in neuropathology

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Introduction: Remnants of topical hemostatic agents are regularly present in neuropathology samples. Generally considered of little diagnostic importance, these hemostatic agents may mimic tumor progression and intracranial abscesses on radiological imaging. **Objectives:** This study illustrates the main macroscopic and microscopic characteristics of commonly applied hemostatic agents. **Materials & methods:** A selection of microscopy slides of sterile hemostatic agents and neuropathology samples from reoperations were reviewed. Results from routine histopathological processing, special stains, and polarized light microscopy were used to document each agent's histological properties. **Results:** Different types of hemostatic agents have distinct features determined by their primary constituents. A classification based on material composition is proposed to aid microscopic identification. **Conclusion:** Background knowledge of hemostatic agents can help the neuropathologist in identifying such materials and ensure accurate reporting of adverse events. **Keywords:** Hemostatic agents – foreign body – neurosurgery – pseudotumor progression – complication

11. OTHER

P063

Activation of the glia limitans in the piriform cortex of Parkinson's disease patients

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Background: Chronic hyposmia is one of the key non-motor symptoms in Parkinson's disease (PD) and frequently begins years before the clinical diagnosis. To date, the exact pathophysiological mechanism underlying hyposmia remains unclear. **Objectives:** We investigated neuropathological changes in the piriform cortex (PC), the first cortical area processing odor information in mammals, comparing PD patients with COVID19 subjects as well as neurological and healthy controls. We focused on standard neuropathological readouts as well as markers for innate immunity, possibly occurring in response to either chronic hyposmia (PD) or acute hyposmia (COVID19). **Methods:** PC samples from 55 individuals were obtained during autopsy and investigated by immunohistochemistry, immunofluorescence, spatial transcriptomics (Visium Platform), combined with in-house-developed, deep learning-based automated analyses. **Results:** PD patients showed Lewy pathology in the PC, co-occurring with other neuropathological processes, especially neuronal and glial tau aggregation. An increased thickness of the superficial glia limitans (GLS) was observed in PD, accompanied by MHC-II-positive macrophages. Additionally, all layers of the PC showed a higher density of Iba1+ microglia. Spatial transcriptomics demonstrated astrocytic activation in the PC of PD patients, distinct from that observed in the cingulate gyrus of PD patients and from corresponding regions in COVID19 brains. **Discussion:** Pathological alpha-synuclein but also p-tau aggregates were identified in the PC of PD as well as selected COVID19

patients. Molecular analyses of human postmortem tissues revealed activation of glial cells in PD in this paleocortical region, especially in the GLS, potentially reflecting a chronically smoldering process furthering neurodegeneration. **Keywords**: Parkinson's disease – olfaction – innate immune system – piriform cortex

P064

Autopsy of the brain modo Rudolph Virchow

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Introduction: Systematic anatomic studies of the brain were introduced during the Renaissance with Leonardo da Vinci (1452–1519), Charles Estienne (1504-1564) and Andreas Vesalius (1514–1564), followed by Franciscus Sylvius (1614–1672) and others. One of their successors was Rudolph Virchow (1821-1902), the founder of modern pathology, who was also involved in the studies of the nervous system. **Objectives, Methods, Results:** He reorganized the procedure and reporting of the general post-mortem examination, including a meticulous autopsy of the brain. When describing the dissection of the brain, Virchow bases his method on a thorough investigation not only of the brain substance but also of the ventricle system, in such a way that the organ after dissection could be analyzed "like a book" bound in the pia mater enveloping the cerebral vessels but also whole structure. The study of the ventricles and meninges was crucial at gross examination as well as under the microscope. **Conclusion:** Brain dissection modo Virchow, however, did not enter the common anatomopathological or neuropathological practice due to its difficult methodology requiring particular skills. **Keywords**: Brain dissection – Rudolph Virchow – autopsy

P066

Brainstem lesions in permanent vegetative state

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Introduction: Vegetative state is associated with widespread damage of cortex, white matter and/or thalamus; brainstem findings are usually absent or include only focal lesions. Functional integrity of the brainstem is considered to be necessary for preservation of arousal/wakefulness, particularly midline structures of the upper pons and midbrain. **Objective:** We present here a case of post-traumatic permanent vegetative state which is characterized by a quite complex damage also at the level of the brainstem. **Patient & methods:** After a car accident a young woman was referred to Neurosurgical Unit in coma. Spontaneous sleep/wake cycle recovered in the following months. Magnetic resonance, performed one month after the trauma, showed injuries of midbrain, thalamus and left frontal lobe. The patient died more than 15 years after the trauma. **Results:** Macroscopic

examination showed generalized atrophy of cerebral hemispheres, cerebellum and brainstem. Partial degeneration of the basal ganglia and severe volumetric reduction of the hemispheric white matter were observed. Wide and bilateral thalamic necrosis was found. Transverse sections of the brainstem showed degeneration of the descending and ascending systems of motor and sensitive fibers, and neuronal loss in the inferior olivary complex, pontine nuclei, red nucleus and substantia nigra. In the midbrain, areas of necrosis with reactive astrogliosis extended from the mesencephalic aqueduct towards the interpeduncular fossa, affecting the periaqueductal grey matter, and the dorsal and median raphe nuclei. **Conclusions:** On the basis of the neuroanatomic description of the damaged areas, we discuss the role of the midbrain reticular activating system and possible plasticity responses in the mechanisms of arousal/wakefulness. **Keywords:** Vegetative state – midbrain – trauma – consciousness

P068/WS14

Eye-tracking in neuropathology: how do experts view histopathological tissue compared to novices

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Introduction: Visual analysis of histopathological tissue is a chore for (neuro-)pathologists. Recent research focuses on automating this process using Machine Learning (ML). These approaches rely on specifically created annotations rather than the pathologists' analysis patterns. **Objectives:** To provide a real-world foundation for ML, eye-tracking is applied to investigate the visual analysis of histopathological slide sections by physicians. **Methods:** A neuropathologist selected 35 slide sections, with some showing neoplastic tissue of Glioblastoma (GBM). A total of 14 participants – 3 experts (6+ years of experience, trained (neuro-)pathologists) and 11 novices (medical students, 2nd–4th year) – were asked to classify the images and highlight the most relevant areas, all while being observed by an eye-tracker. We developed our own application capable of presenting pictures sequentially, allowing for classification and highlighting areas. All participants received the same introduction to GBM classification. **Results:** Novices were less likely to classify image sections as neoplastic (25% vs. 52.2% for experts). No obvious analysis patterns were detected. Of the four major artifacts (two folds, two tissue accumulations), only one artifact area was focused by a single expert (8%), while on average, 43% of novices focused on each artifact. Two novices had their main focus on artifacts. Novices required on average 12:15min (+/- 2:41min) for analysis while experts required 4:34min (+/- 1:37min). **Conclusion:** Eye-tracking was used to compare the visual analysis behavior of histopathological tissue between experts and novices. Novices were more likely to base their analysis on artifacts. No common patterns could be found in either group. **Keywords:** Eye-tracking – histopathology – glioblastoma – visual analysis

P069

On unexpected findings in brain autopsies

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Introduction: A worldwide trend to abstain from post mortal examinations can be observed. However, many unexpected or previously not diagnosed lesions are often encountered during autopsies, especially in central nervous system. **Objectives:** The aim of our study was to estimate how many relevant undiagnosed lesions can be found in post mortal examinations of the central nervous system (CNS) and to prove the importance of autopsies in modern medicine. **Patients & methods:** We reviewed reports from 256 autopsies performed between April 2022 and March 2024 at the Pathology Department of the University Hospital in Augsburg, Germany, searching for relevant findings that have not been diagnosed premortal clinically or radiologically. Fetal (36) and partial autopsies omitting brain examination (24) were excluded from the study. Relevant findings were defined as not age-related and not-perimortal changes. **Results:** In 118 patients (44,9%), a total of 162 relevant CNS pathologies were diagnosed by brain autopsy, of which 77 (47,5%) were clinically under- or undiagnosed and seven (4,3%) could be declared as cause of death. The most common unexpected findings were vascular diseases including cerebral infarcts and hemorrhages (34 cases, 20,9%), however some rather rare conditions (e. g. IVLBCL, central toxoplasmosis, meningocerebral angiodysplasia) as well as undiagnosed neurodegenerative disorders (Parkinson's and Alzheimer's disease) were described. **Conclusion:** Our study confirms that a relevant number of undetected lesions can be found in post mortal examination, despite of progress in modern diagnostic. These findings underline the role of systematic examinations of CNS in autopsy studies. **Keywords:** Autopsy – morbidity – vascular disease – brain neoplasm – infective disease

P071

Three-dimensional reconstruction of neurons and connectivity patterns in the human spinal cord

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Introduction: Traditional histopathological staining methods are performed on specimens with a thickness of 2-5 µm, providing a two-dimensional cross-sectional view of neurons that is largely limited to the soma. The exact morphological classification of neuronal subtypes, their dendritic receptor expression, and the identification of CNS connectivity patterns, however, require a three-dimensional visualization of neurons including their dendritic arborization and branching patterns. A staining method that enables the visualization of whole neurons is the Golgi-Cox impregnation technique, which can be combined with immunofluorescent antibodies targeting specific proteins. Recent advancements in microscopy imaging and computational postprocessing techniques have further facilitated the acquisition of high-resolution images of neurons. **Objectives:** To provide a three-dimensional visualization of single neurons in the spinal cord and their connectivity patterns, as well as the dendritic spines that are crucial for synaptic plasticity. **Materials & methods:** In this study, human post-mortem spinal cord tissue will be used for the Golgi-Cox impregnation of spinal neurons

in combination with tissue clearing and immunofluorescence staining of catecholaminergic receptors. Images will be acquired using confocal microscopy. **Results:** Single-impregnated neurons including their soma and dendritic architecture will be segmented and subsequently reconstructed in three dimensions using AI- and deep learning-based models. The resulting images will be compiled into a comprehensive cyto- and dendroarchitectonic atlas of the lumbar spinal cord. **Conclusion:** This staining technique can be applied to various regions of CNS tissue and offers numerous applications in basic research, providing a deeper understanding of healthy neuronal function and potentially offering new insights into neurological disorders associated with neurodegeneration and synaptopathy. **Keywords:** Golgi impregnation human spinal cord – three-dimensional neurons – confocal reflection microscopy

P149

Clinical pathology and genetic characteristics of late onset methylmalonicemia induced by infection

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Objectives: Immunological imbalance induced by COVID-19 initiated acute onset of late-onset methylmalonicemia (MMA). **Patients & methods:** A 56-year-old male presented tremor, cognitive decline, and slowed speech after COVID-19 infection. Cranial MRI showed cerebral atrophy and bilateral periventricular white matter T2WI hyperintensities which were line-like enhanced on the enhanced MRI. There was “Black Pepper” enhanced on the enhanced cervical MRI. Cerebrospinal fluid (CSF) protein was 2927mg/L and white cells were 155×10^6/L. But on the fifth day he was in a silent state, unable to eat or swallow on his own. At first, post-COVID-19 syndrome was diagnosed. He was treated with glucocorticosteroids. Later, he was in abnormal mental behavior. **Results:** Blood homocysteine (Hcy) 98 μmol/L. The level of Methylmalonic acid was higher than normal. Whole exome testing suggested MMACHC mutation genes c.1A>G and c. 482G>A. Brain biopsy showed cerebral leukoedema, vacuole degeneration, glial cell hyperplasia, interstitial and local perivascular lymphatic cuff formation, and demyelination. **Conclusion:** Infection further exacerbated the immune imbalance of the patient, bringing out the symptoms of MMA. White matter lesions might be due to methylation dysfunction and toxic effects of abnormal fatty acid metabolites. Hematuria metabolism screening and genetic testing could help early diagnosis and guide treatment. **Keywords:** Infection – late-onset methylmalonicemia – clinical pathology

12. Prion diseases

P074

Chronic traumatic encephalopathy and prion disease: First confirmed case in an elite soccer player in Spain and neuropathological screening study.

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Introduction: Chronic traumatic encephalopathy (CTE) is associated with soccer and linked to neurodegenerative diseases. However, its association with prion diseases or reports in soccer players in Spain are scarce. **Objectives:** To describe the first confirmed case of CTE in Spain in an elite professional soccer player and to perform neuropathological screening for CTE in a cohort from the Catalonia reference center for prion diseases. **Methods:** A clinical and neuropathological examination was conducted on a former elite professional soccer player with prion disease. Screening of the hippocampus and frontal cortex with phosphorylated tau staining was performed in 212 subjects with CJD from our brain bank cohort. Additional analyses were conducted in suggestive cases, classifying CTE pathology based on NINDS/NIBIB criteria. **Results:** The patient developed dementia in the 7th decade of life, with rapid decline 6 years later, leading to death within 3 months. Postmortem analysis confirmed prion disease (MM/MV1) and CTE lesions in the frontal cortex with atypical tauopathy. Screening identified CTE in 3 of 212 cases, including the soccer player, all at high pathological stages. Limited clinical data on these cases precluded confirmation of their sporting habits or repetitive head impacts, though they were not professional soccer players. **Conclusion:** CTE was identified in a professional soccer player with prion disease, representing 1.4% of prion disease cases in the cohort. While no evidence supports repeated head impacts as a predisposing factor for iridopathies in these cases, the presence of CTE suggests a potential association that warrants further investigation. **Keywords**: Chronic traumatic encephalopathy – prion diseases – soccer

13. Target therapy in neuro-oncology

P075

5-methylcytosine as a marker of epigenetic effect in the treatment of glioblastoma using repurposed drugs

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Introduction/Objectives: Glioblastoma (GBM) is currently being researched by many drug developers due to its infiltration nature, which necessitates additional locoregional (radiotherapeutic) and

systemic (with temozolomide, TMZ) treatment besides surgery. The majority of patients receive parallel therapy for brain edema (dexamethasone, DEX), with some also receiving medications for epilepsy (valproic acid, VPA) and diabetes (metformin, MF). Epigenetic events play a crucial role in gliomagenesis. The best-characterized epigenetic mark is 5-methylcytosine (m5C) in DNA. It has been demonstrated that oxidative DNA damage and increased tumor malignancy correlate with decreased total DNA methylation. Considering this, we opted to analyze the influence of the most widely used drugs in GBM on total DNA methylation. **Methods:** Using the nucleotide post-labelling method, we analyzed the total amount of m5C in DNA of GBM cell lines treated with TMZ, DEX, VPA, MF, and their combinations. **Results:** We adjusted the TMZ, VPA, and MF doses to those achieved in the brain during treatment. We observed dose-dependent changes in the total DNA methylation in GBM cell lines. VPA alone produced a clear dose-dependent increase in total DNA methylation, while MF decreased the level of m5C. The influence of TMZ was concentration-dependent. The combination of both drugs with TMZ caused DNA demethylation. **Conclusion:** Total DNA methylation alterations in GBM cell lines exposed to TMZ, VPA, and MF treatments indicate a novel epigenetic mechanism of action, highlighting potential clinical implications for optimizing dosages and combinations in GBM therapy. Research supported by OPUS 19 (2020/37/B/NZ5/03249) from the National Science Center, Poland. **Keywords:** Glioblastoma – epigenetics – DNA methylation – 5-methylcytosine – oxidative stress – drug repurposing

P076

CNS-tumor patients within the IMPRESS-Norway trial

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Introduction: IMPRESS-Norway is an ongoing, nation-wide, precision medicine trial for patients with progressive cancer. In this investigator-initiated, prospective, open-label, non-randomized combined

basket and umbrella trial, patients are enrolled into parallel treatment cohorts. All 24 drugs available in IMPRESS-Norway are regulatory approved. **Objectives:** The primary objective in the study is clinical benefit after 16 weeks of treatment; defined as complete response, partial response or stable disease. Here, we report patients with CNS-neoplasms included in IMPRESS-Norway profiling and treatment phases. **Patients & methods:** The study uses publicly available advanced diagnostics and follows the recently updated EANO-guideline on rational molecular testing for targeted therapy selection. Patients with identified biomarkers matching available drugs are referred by the national molecular tumor board (nMTB) for inclusion in the treatment phase. **Results:** 216 patients with CNS-neoplasms had been included in molecular profiling and completed evaluation in nMTB by 31.12.2024. 177 patients were diagnosed with adult type diffuse gliomas. Several newly recognized entities, e.g. high-grade astrocytoma with piloid features, were diagnosed in the remaining patients. We identified actionable targets in 50 patients and initiated therapy in 27 patients. Treatment is ongoing in 5 patients, including three glioblastoma patients with complete response (week 156), partial response (98% tumor size reduction at week 81) and stable disease (week 81). **Conclusion:** Molecular alterations indicating actionable targets available in IMPRESS-Norway were detected in 23% of patients with CNS-neoplasm. Response assessment contributes to refinement of the ESMO scale for clinical actionability of molecular targets (ESCAT) in CNS-tumors. **Keywords:** Precision medicine – glioblastoma – biomarker – targeted therapy – oncology – neuropathology

P077

The role of chemokine receptor expression in the progression of brain metastases in different cancer types- A retrospective study

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Background: Lung cancer, renal cell carcinomas, colorectal carcinoma and malignant melanomas are the most common cancers in industrial countries and the leading cause of cancer-related mortality. Especially the development of brain metastases is accompanied by poor prognosis. Chemokines and their receptors play a key role in the formation of metastases, but also in disease progression. **Methods:** This thesis aimed to find out if the chemokine receptor expression in Non-Small-Cell Lung Cancer (NSCLC), Clear cell renal carcinomas (RCC), Colorectal Carcinomas (CRC) and Malignant Melanomas (MM) are different between the primary tumors and brain-specific metastases. Differences in the expression of the most well-characterized chemokine receptors (CCR1- 10, CXCR1-7, XCR1 und CX3CR1) were detected by RT-qPCR. **Results:** The mRNA expression profile for some chemokine receptors is different in brain-specific metastases of NSCLC compared to the primary tumors. Also, in RCC and CRC there is a difference between the expression of specific CCR in primary tumors and brain metastases. Even Chemokine expression patterns in melanomas are different to those in brain specific metastases. Furthermore, the chemokine profile is significantly different in primary melanomas compared to their surrounding skin tissue. **Conclusion:** New insights in this field may yield new concepts in developing targeted therapies for primary carcinomas and especially for brain metastases. **Keywords:** Brain metastases – chemokine receptors – targeted therapy – immune therapy

14. Tumors - gliomas

P078

A rare and unusual subtype of a pediatric high-grade glioma with epithelioid SEGA-like morphology and evidence of MAPK/TSC1/mTOR pathway activation; a small case series

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Introduction: High-grade gliomas (HGG) arise in any central nervous system location. They occur in adults and children with a poor prognosis (20.8% 5-year survival for those aged 0-19 years and 21.9% for those aged 20-44 years). This is better than older adults; those aged 45-54 and 55-64 years have a 5-year survival of 9.3% and 5.9%, respectively. Published literature reports a rare adult-specific periventricular glioblastoma showing SEGA-like morphology with driver mutations in NF1 and MTOR, and TSC1 subclonal mutations, suggesting up-regulation of the MAPK/TSC1/mTOR pathway. We present a case series of pediatric cases demonstrating similar features. **Clinical case:** The patients were aged 3-12 years (2 male, 1 female). The tumors were located in hemispheric locations. All showed appearances favoring an HGG on MRI imaging. The histological features were those of HGGs with an epithelioid/giant cell component. DNA methylation profiling classified the tumors as 'Glioblastoma_IDH-wildtype_mesenchymal type' (calibrated scores of 0.38, 0.64, 0.84). DNA panel sequencing detected variants in NF1, TP53, TSC1, TSC2, and ATRX across these cases; a TP53 germline mutation was detected by WGS in one patient. Copy number profiling showed multiple changes, including gains of chr7 and a CDKN2A/B deletion in one case. **Conclusions:** NF1 and TSC1/2 variants alongside focal giant cell morphology suggest these tumors resemble the described periventricular glioblastoma with epithelioid SEGA-like morphology, illustrating that these tumors also occur in the pediatric setting. It represents an opportunity to further explore the relationship between phenotype and molecular characteristics in HGG. **Keywords:** High-grade glioma – pediatrics – TSC1/2 mutations – novel tumor

P079

Adult primary leptomeningeal gliomatosis with granular cell morphology: A biopsy and autopsy case report with molecular characterization.

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Introduction: Adult primary leptomeningeal gliomatosis (PLG) is a rare and aggressive glial tumor that primarily involves the leptomeninges without an identifiable parenchymal mass. Recent molecular analyses have classified it alongside glioblastoma, IDH-wildtype, CNS WHO grade 4. The rare granular cell variant has not been previously reported in PLG. **Clinical case:** A 77-year-old woman presented with progressive leg weakness and lumbar pain. MRI revealed diffuse spinal cord and cauda equina enhancement, raising the differential diagnosis of neurosarcoidosis, tuberculous meningitis and leptomeningeal carcinomatosis. CSF showed elevated protein, low glucose and pleocytosis, but was negative for tumor cells and microbiological tests. A biopsy of the cauda equina nerve roots confirmed a diffuse glial neoplasm with granular cell morphology. The tumor was IDH- and H3-wildtype and harbored a TERT promoter mutation, CDKN2A homozygous deletion and chromosome 7 gain only. An integrated diagnosis of Glioblastoma, IDH-wildtype, CNS grade 4 was made. Palliative treatment was decided and the patient died of disease nine days after the diagnosis. Autopsy revealed extensive leptomeningeal dissemination throughout the brain, spine, and cauda equina, with focal secondary intraparenchymal tumor spreading, confirming the diagnosis of PLG with focal granular cell morphology. DNA methylation array profiling with the Heidelberg classifier (version 12.8) on autopsy FFPE material yielded a highest confidence score of 0.22 for Glioblastoma, IDH-wildtype, mesenchymal subtype. Results from repeat analysis on the biopsy specimen are pending. **Conclusion:** This case underscores the diagnostic challenges of PLG, which can mimic infectious or inflammatory conditions like sarcoidosis, particularly when exhibiting granular cell morphology. **Keywords:** Primary leptomeningeal gliomatosis – gliomatosis – glioblastoma – granular cell glioblastoma

P080

Adult-type diffuse high-grade glioma, IDH-wildtype, subtype E" in a pediatric patient with CMMRD syndrome: A new WHO entity?

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Background: The 2021 WHO Classification of CNS Tumors introduced more than 150 histologically and/or molecularly distinct adult and pediatric entities. Since its publication, many potential new tumor types have been proposed based on DNA methylation profiling. CMMRD syndrome associated with MMR deficiency has been typically associated with the « Diffuse pediatric-type high grade (HG) glioma, RTK1 subtype". Here, we report the case of a potential new CNS tumor entity that could specifically be associated with CMMRD syndrome. **Clinical case:** A 12-year-old boy followed for a CMMRD syndrome presented worsening headaches and transient vision loss. The MRI revealed a left temporal tumor with enhancement and mass effect. A large but incomplete resection confirmed a polymorphous, spindle-cell shaped HG diffuse glioma, with oligodendroglioma and PNET-like features. MSH6 protein loss of expression was found. Molecular analyses identified ATRX, TP53, POLE, and NF1 mutations and CDK4/MET amplification while DNA methylation profiling classified the tumor as "Adult-type diffuse HG glioma, IDH-wildtype, Subtype E". A few weeks later, tumor recurrence led to a second

near-total resection but unfortunately quickly progressed just after the second surgery leading to palliative radiotherapy. **Conclusion:** In the literature, no specific clinico-pathological and molecular characterization is available regarding the "Adult-type Diffuse HG Glioma, IDH-wildtype, Subtype E". This potential new entity seems to harbor polymorphous morphology, very aggressive clinical evolution and could be associated with CMMRD syndrome in the pediatric population. Further investigations are crucial to provide a better understanding of this entity and its clinical implication. **Keywords:** Pediatric CNS tumors – glioma – adult-type diffuse high-grade glioma – DNA methylation – WHO classification

P081

Adult-type diffuse high-grade glioma, subtype F: The return of the gliomatosis cerebri pattern

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Background: The 2021 WHO classification of CNS tumors classification was updated based on radiological, morphological and molecular data and some entities previously considered to be distinct have been removed. This is the case for Gliomatosis Cerebri (GC), which was considered rare but independent clinico-pathological entity, remains as a spread pattern of diffuse gliomas. Here, we report on the case of a potential new CNS tumor entity specifically associated with a GC pattern. **Clinical case:** The MRI, performed to a 56-year-old man who presented with episodes of tonico-clonic seizures and loss of consciousness, revealed a bi-hemispheric, supra-tentorial, multifocal and infiltrative lesion which was initially suspected to be an inflammatory process rather than a tumor, given the absence of contrast enhancement. The surgical biopsy revealed a high-grade (HG) glioneuronal tumor with glioneuronal features, a "DNET-like" growth pattern and a TERT promoter mutation. Finally, DNA methylation profiling led to the definitive diagnosis of Adult-type diffuse HG glioma, IDH-wildtype, subtype F. Despite the treatments provided for his intracranial hypertension, the patient died just one week after the biopsy. **Conclusion:** Very few data are available regarding the Adult-type diffuse HG gliomas, subtype F which seem to exhibit highly heterogenous histological features and an unknown prognosis. This potential new entity brings the "GC spread pattern" back into focus after it had been progressively abandoned. Updating the classification to incorporate the newly identified subtypes associated with this pattern is essential. **Keywords:** Gliomatosis cerebri – glioma – adult-type diffuse high-grade glioma – DNA methylation – WHO classification

P082

Astroblastoma with an unusual gene fusion: Case report and discussion

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Introduction: Astroblastoma MN1-altered became part of the WHO classification of CNS tumors in 2021 and has a distinct DNA methylation profile. While essential diagnostic criteria include the presence of the MN1 alteration (a gene fusion with BEND2), a few other cases have been described sharing the methylation pattern. **Clinical:** We present the case of a 34 year old female patient with a recurrent tumor in the parietal and temporal-insular location. It was resected in July 2023. She underwent 2 previous resections in 2017 and 2019, diagnosed as ependymoma. In 2023 tumor morphology consisted of small cells with rounded nuclei forming chords and ribbons, with occasional perivascular rosettes. Mitotic activity was high and there were small necrotic areas. The consistent absence of glial markers (GFAP, S100) and high proliferation index were noted. Diffuse membranous CD99 and EMA suggested a differential with ETMR (ependymoblastoma type) so evaluation for characteristic genetic alterations was recommended. The tumor recurred so the patient underwent another resection in January. In April 2024, NGS-sequencing revealed the EWSR1::BEND2 fusion. While few similar cases are reported in the 2021 classification (spinal location) it is currently recommended to use the NEC designation. A methylation profile performed in July 2024 indeed classified the tumor as astroblastoma, MN1-altered (calibrated score 0.99) despite the lack of the characteristic fusion. **Conclusion:** To our knowledge 8 other EWSR1::BEND2 fused astroblastomas have been reported, 2 being supratentorial. Since there are differences concerning treatment of this entity, a further understanding of its pathogenesis may be necessary. **Keywords**: Pediatric glioma – low grade glioma – ependymoma – extra ventricular – supratentorial ependymoma – ZFTA fusion – diffuse glioma

P083

Astroblastoma with MN1-CXXC5 fusion: Clinical, histological and molecular characteristics of two pediatric cases

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Background: Pediatric brain tumors are challenging to diagnose and treat due to their heterogeneous nature. Tumors with MN1::CXXC5 gene fusion are exceedingly rare and molecularly distinct from other MN1-altered tumors, such as astroblastomas. **Objectives:** We report two pediatric cases of brain tumors with MN1::CXXC5 gene fusion to contribute further knowledge regarding this rare subgroup's histological, molecular, and clinical characteristics. **Methods:** Case #1 is an 8-year-old male with a right fronto-parietal lesion. Case #2 is a 9-year-old male with asthenia, frontal headache, and acute right upper limb weakness followed by hemiparesis. Both underwent radical excision. Histopathological examination, fluorescence in situ hybridization (FISH), and DNA methylation profiling were performed.

Results: Histopathology of both cases showed a high-grade glioma-like tumor with a papillary growth pattern around hyalinized blood vessels, necrosis, and microvascular proliferation. The tumors were positive for GFAP, Olig2, EMA, and SMA. FISH analysis revealed MN1 break-apart, and methylation profiling classified them as "neuroepithelial tumor, MN1::CXXC5-fused" (score 0.99). Both patients received radiotherapy and chemotherapy. Case #1 experienced recurrence with cerebellar and leptomeningeal involvement after 1 year, while Case #2 showed no recurrence after 6 months. **Conclusions:** The MN1::CXXC5 fusion is a rare genetic alteration linked to neural development and cell signaling. Both cases demonstrated similar morphological, genetic and epigenetic features, suggesting the potential existence of a distinct high-grade glioma subgroup. Despite aggressive treatment, recurrence occurred in Case 1, highlighting the potential for relapse in these tumors. Further research is needed to better understand their behavior, improve prognosis and treatment strategies. **Keywords:** MN1 – high grade glioma – pediatrics – astroblastoma

P084

BATMAN: breakpoint adaptive targeting alongside methylation analysis on nanopore

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Introduction: Structural rearrangements are increasingly understood to significantly alter the prognosis of a wide range of CNS tumors. For example, co-deletion of CDKN2A/B is a hallmark of high-grade astrocytoma, oligodendroglioma and meningioma. Despite their clinical importance, detection of intra-chromosomal rearrangements is challenging using short-read sequencing and copy number variations (CNV) are commonly inferred from methylation array. Such testing has limited resolution to detect small, intra-chromosomal CNV calls with high confidence. **Objectives:** Nanopore technology is an emerging technique in CNS diagnostics, enabling targeted long-read sequencing alongside methylome classification. However, current implementations of adaptive targeting are restricted to an a priori defined set of key driver oncogenes. In contrast, genomic breakpoints underpinning SVs are unique to individual patients and are thus challenging to sequence at high coverages. **Methods:** Here, we present BATMAN, a development of the ROBIN diagnostic tool that enables real-time enrichment of patient-specific genomic breakpoints. BATMAN infers genomic breakpoint loci during sequencing and dynamically adapts the target panel in real-time. **Results:** We present the performance of BATMAN on CNS tumors harboring a variety of CNVs. We demonstrate the potential to generate long-reads spanning deletion of CDKN2A/B in high-grade astrocytoma, oligodendroglioma, and glioblastoma. Furthermore, we demonstrate the ability of BATMAN to resolve complex rearrangements such as chromothripsis events and accurately infer genome-wide copy number variation load, an emerging prognostic biomarker. **Conclusion:** BATMAN enables the sequencing of long reads spanning breakpoints at high coverages, enabling the robust detection of clinically relevant SVs. **Keywords:** CNV – nanopore – glioma – structural variation – CDKN2A/B

P085

Characterization of the surgical margin in glioblastoma recurrence using single-cell spatial transcriptomics

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Background: Glioblastoma, the most aggressive brain cancer, is treated with surgery, chemotherapy, and radiation. Despite these efforts, recurrence is inevitable and often occurs near the primary tumor's resection margin, where treatment effects are most pronounced. This region, characterized by fibrosis and inflammation, contains both malignant and non-malignant cells that may interact and drive tumor progression. **Objectives:** To investigate the spatial organization, molecular profiles, and cellular dynamics of the surgical margin of recurrent glioblastomas to gain insights into mechanisms underlying treatment resistance and progression. **Methods:** We used the Xenium Analyzer (10x Genomics) to spatially profile 5,000+ genes in tissue sections at single-cell resolution. The study included 40 glioblastoma patients, IDH-wildtype, with recurrence. Areas from the surgical margin, tumor-dense regions, and matched primary tumors were examined. Computational analyses were applied to classify malignant and non-malignant cells within the surgical margin, analyze their gene expression patterns, cellular compositions, and interactions, and compare these features to other tumor regions. **Results:** The surgical margin exhibited spatial heterogeneity linked to time-to-recurrence, with tumor cells showing increased expression of autophagy-related genes and a shift away from proneural and proliferative states. Non-malignant cell populations, including T cells, a specific endothelial state, and M2-like macrophages, were spatially correlated and displayed cytokine-receptor interactions indicative of an active inflammatory environment in early recurrences. **Conclusion:** Our findings reveal unique cellular dynamics within the surgical margin, suggesting it could contribute to the survival of treatment-resistant tumor cells. Ongoing analyses aim to further explore these features and their role in glioblastoma recurrence. **Keywords:** Glioblastoma – recurrence – surgical margin – single-cell spatial transcriptomics – tumor microenvironment – treatment resistance

P086

Clinico-pathological study of a monoinstitutional series of lower-grade gliomas: impact of molecular markers based on the new WHO CNS5 classification

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Introduction: Low-grade gliomas (LGGs) were classified by WHO as grade 2-3 tumors; the 2021 update included primarily molecular characteristics: homozygous deletion (HD) of CDKN2A/B gene which assigned grade 4 and 3 to IDH-mutant astrocytomas and oligodendrogliomas; and IDH wild type, TERT

promoter mutations, EGFR amplification or chromosomes 7 and 10 gain/loss which characterized glioblastomas. **Objectives:** The study was conducted to identify prognostic markers that can be implemented in routine clinical practice to enhance diagnostic process and enable timely advanced molecular analyses. **Materials & methods:** We carried out a retrospective study in a single-institution cohort of 158 LGGs patients. We assessed by immunohistochemistry the expression of PDGFRa, p16, CDK4, and to evaluate the state of CDKN2A/B and the status of 9p chromosome, MLPA and microsatellite analysis were performed. In addition, we also investigated CDK4 amplification by copy number variation (CNV). **Results:** Oligodendrogliomas showed higher p16 expression than astrocytomas, indicating its prognostic value for overall survival (OS). We found a correlation between loss of heterozygosity (LOH) on chromosome 9p, reduced p16 expression, and HD of the CDKN2A/B gene, mainly in grade 3 astrocytomas. Additionally, gene amplification and overexpression of CDK4 were noted with 9p LOH, suggesting its prognostic role. PDGFRa expression was higher in oligodendrogliomas but did not correlate with the outcome. **Conclusion:** In conclusion, biomarkers such as CDK4 and p16 demonstrate a significant potential in facilitating early diagnosis within the framework of molecular analyses, which often require extended timeframes. **Keywords:** Lower-grade gliomas – immunohistochemistry – CDKN2A/B – CDK4 – p16 – prognostic markers

P087

Correlation of CLOCK and BMAL1 gene expression with PD-L1 immunohistochemical expression in gliomas and evaluation with histopathological parameters

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Objectives: Gliomas are the most common tumors among central nervous system malignancies. In high-grade gliomas, survival is short, and the prognosis is poor. Studies have shown that the circadian rhythm plays a significant role in tumor cell proliferation, invasion, response to chemotherapy, and immune-suppressive mechanisms in the tumor microenvironment. For this reason, chrono chemotherapy has become an intriguing area of research. **Methods:** The expressions of CLOCK(circadian locomotor output cycles kaput) and BMAL1(brain and muscle aryl hydrocarbon receptor nuclear translocator-like protein 1) were compared at different times of the day in 72 cases. Immunohistochemical evaluation of PD-L1(programmed death-ligand 1) expression was conducted to assess its correlation with CLOCK and BMAL1 expression, and its relationship with other prognostic parameters was investigated. **Results:** In IDH-wild type glioblastomas, significantly higher PD-L1 TPS (tumor proportion score) was observed compared to IDH-mutant astrocytomas. Significantly higher BMAL1 expression levels were found in tumor tissues compared to control tissues. CLOCK expression was higher in the morning hours in both IDH-mutant astrocytomas and IDH-wild type glioblastomas, and in IDH-mutant astrocytomas, expression decreased with age. Higher CLOCK and BMAL1 expression levels were observed in p53 mutant IDH-wild type glioblastomas. PD-L1 expression was significantly higher in glioblastomas, with higher expression observed in the morning hours. Additionally, BMAL1 expression was higher in tumors with a TPS score of 2(≥50%). **Conclusion:** The findings suggest that chemotherapy administered in the morning hours may be more effective, and that the circadian rhythm effect may be more pronounced, particularly in younger individuals. Furthermore, considering the circadian rhythm in immunotherapy and p53-targeted treatments for IDH-wild type glioblastomas could potentially alter treatment efficacy. **Keywords**: BMAL1 – circadian rhythm – CLOCK – glioma – PD-L1

P088

Cross-methodological analysis of the tumor microenvironment in primary and recurrent glioblastoma

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Introduction: The glioblastoma (GBM) tumor microenvironment (TME) consists of diverse cellular populations, including tumor, immune, and stromal cells, contributing to its protumorigenic and immunosuppressive nature. The TME in recurrent GBM (rGBM) undergoes significant changes compared to primary tumors. Various in-situ and in-silico approaches exist to assess immune cell populations, each offering distinct advantages and limitations in characterizing the TME. **Objectives:** This study aims to highlight the critical distinctions between in-situ and in-silico methods for analyzing the GBM TME in primary and recurrent tumors. **Materials & methods:** A cohort of 36 matched primary and recurrent GBM cases (72 samples) was analyzed. TME assessment included cellular proportion quantification using in-situ methods—conventional and AI-driven immunohistochemistry (IHC), multiplex immunofluorescence (IF)—and in-silico approaches, such as DNA methylation profiling and RNA deconvolution. **Results:** Cross-methodological analysis showed high concordance in monocyte/macrophage populations, whereas B-cell consistency was lower, particularly in DNA methylation and in all three in-situ approaches (IHC-conventional, IHC-pipeline, and multiplex-IF). Longitudinal investigation revealed an increased dominance of immunosuppressive macrophages and CD8-positive T cells in rGBM. DNA methylation analysis identified shifts in CNP, MGMT-promoter, and DNA methylation subclasses, while gene expression profiling showed distinct patterns: primary tumors exhibited upregulation of genes linked to immune invasion and tumor progression, whereas recurrent tumors displayed signatures of neuroinflammation, ECM remodeling, and therapy resistance. **Conclusion:** rGBM undergoes profound immune and molecular remodeling, underscoring the need for tailored therapeutic strategies targeting immune suppression and subtype-specific vulnerabilities. Our findings highlight the importance of integrative multi-modal analyses to refine treatment strategies and improve clinical GBM management. **Keywords:** Recurrent glioblastoma – tumor microenvironment – AI-driven immunohistochemistry – longitudinal analysis – cellular proportion quantification

P089

Diffuse gliomas in adults slipping through the cracks of the WHO classification of CNS tumors (5th ed.): 3 examples

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Introduction: Some diffuse gliomas in adults do not fit in one of the existing WHO CNS5 diagnostic categories or behave differently as expected. Here we discuss 3 examples. **Cases:** A 71-year-old-male presented with a fronto-insular, histologically low-grade, diffuse astrocytic lesion, meeting criteria for *Glioblastoma IDH-wildtype* (GBM) due to the presence of a *TERT* promotor mutation. DNA methylation profiling (DNAMP) provided no suggestion for a diagnosis with a high score. Our diagnosis was: 'Diffuse, histologically low-grade, IDH-wildtype glioma, *TERT*p mutation only, not elsewhere classified (NEC)'. A 70-year-old-female presented with a mesiotemporal, histologically low-grade, diffuse astrocytic lesion, IDH-wildtype that did not meet the histomolecular diagnostic criteria for GBM. DNAMP revealed almost complete loss of chromosome 10, but no gain of chromosome 7 and did not suggest a class with a reliable score. Our diagnosis was: 'Diffuse, histologically low-grade astrocytoma, IDH-wildtype, NEC'. A 60-year-old male presented with a frontal diffuse astrocytic lesion, IDH-wildtype, not meeting the histomolecular criteria for GBM, but with many giant monstruous cells and frequent mitoses. The patient appeared to have a *POT1* germline mutation. Our diagnosis was: 'Diffuse, high-grade glioma, IDH-wildtype, NEC'. **Conclusions:** 1) Caution should be exercised when assigning WHO grade 4 to '*TERT* promoter only', histologically low-grade diffuse gliomas, as they may exhibit intermediate-grade behavior. 2) The prognostic impact of incomplete chromosome 7 gain and/or chromosome 10 loss is less clear and requires cautious interpretation. 3) Difficult-to-classify diffuse gliomas may be caused by unusual oncogenesis; for tumors with multiple giant tumor cells an underlying genetic tumor syndrome should be considered. **Keywords**: Diffuse glioma – WHO classification – TERT promoter mutation-only – glioblastoma – not elsewhere classified – POT1 mutation

P090

Diffuse midline glioma, H3 K27 altered case series: Including rare cases and molecular insights

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Introduction: Diffuse midline glioma (DMG) is a highly aggressive central nervous system tumor predominantly affecting pediatric and young adult patients and classified in the group of "Pediatric Type High Grade Diffuse Glial Tumors". This study aims to evaluate the clinical, demographic, and molecular characteristics of DMG cases diagnosed at our institution, with a focus on rare cases and long-term survivors. **Methods:** A retrospective analysis was conducted on 166 DMG patients. Demographic data was analyzed. Molecular profiling, long-term survival, and rare presentations were also evaluated. **Results:** Among the 166 cases, 114 (68.7%) were pediatric patients, while 53 (31.3%) were adults. The mean age was 17.75 years, with a median age of 12 years. Gender distribution was nearly equal, with 84 female (50.6%) and 82 male (49.4%) cases. Rare cases included two patients with vertebral bone metastases and seven patients of advanced age (>50 years). Long-term survival was observed in one case exceeding five years and four cases exceeding four years. One case exhibited hemispheric tumor localization outside the midline. Molecular analysis via next-generation sequencing (NGS) was performed on 85 cases. Among these, in addition to H3 K27M mutations; one patient harbored an IDH2 mutation, three cases exhibited a BRAFV600E mutation, eight cases had an EGFR mutation, and within this series three cases showed EZHIP overexpression. **Conclusion:** Our study confirms that DMG predominantly affects pediatric patients, with a nearly equal gender distribution.

Additionally, rare cases with vertebral metastases, advanced age, hemispheric localization, and long-term survival highlight the heterogeneity of DMG. Molecular findings further contribute to understanding the tumor's genetic landscape. Further research is needed to explore prognostic implications and therapeutic approaches for these rare presentations. **Keywords:** Diffuse midline glioma – H3 K27 – molecular insight

P091

Diffuse pediatric-type high-grade glioma, H3 wild-type, IDH wild-type, with MYCN amplification: A case report

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Introduction: Diffuse pediatric-type high-grade gliomas (H3 wild-type, IDH wild-type) are classified as CNS-WHO Grade4 tumors. They are categorized into three groups based on their methylation profiles: pHGG-RTK1, pHGG-RTK2, and pHGG-MYCN. **Clinical case:** A 10-year-old male patient with no significant medical history presented with headache and vomiting. MRI revealed a lesion in the left supramarginal gyrus, prompting surgical excision. Histopathological examination showed a tumor with two morphologies: hypercellular regions with primitive-appearing cells and hypocellular areas with glial cells. Perivascular pseudorosettes were observed in the hypercellular areas, where the cells exhibited marked atypia, pleomorphism, and necrosis. The hypocellular areas featured multinucleated giant cells with bizarre nuclei. In 10HPF, 34 mitoses were identified. The differential diagnosis included atypical teratoid/rhabdoid tumor (ATRT), CNS tumor with BCOR internal duplication, CNS neuroblastoma with FOXR2 activation, embryonal tumor with multilayered-rosettes, cribriform neuroepithelial tumor, high-grade supratentorial ependymoma, and pediatric-type diffuse high-grade gliomas. Immunohistochemical analysis revealed diffuse GFAP positivity in the hypocellular regions, partial perivascular positivity in the hypercellular areas, and diffuse Olig-2 positivity. Ki-67 index was 85%. Synaptophysin and BCOR were negative, with no loss of INI-1 expression. Focal EMA and nonspecific L1CAM staining were observed. FISH analysis showed no amplification of MYCN, MYC, or EGFR. NGS detected mutations in TP53 and NF1. Methylation analysis classified the tumor as a diffuse pediatric-type high-grade glioma, H3 wild-type, IDH wild-type, with MYCN amplification. **Conclusion:** H3 wild-type, IDH wild-type diffuse pediatric high-grade gliomas are aggressive tumors requiring careful differentiation from other embryonal and high-grade gliomas. Methylation profiling remains the gold standard for accurate diagnosis. **Keywords:** Glioma – pediatric – methylation

P092

DNA methylation profiling of CNS tumors, real-world experience from King Hussein cancer center

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Introduction: DNA-methylation-profiling is increasingly used in the classification of CNS tumors. **Objectives:** Share a one-year real-world experience of CNS methylation profiling testing in CNS tumors, from the setting of a cancer center in a resource-limited setting. **Patients & methods:** 240 CNS tumors from 220 patients were examined, with a median age of 23 years. Calibrated score (CS) ≥0.9 was considered for a definite diagnosis. Concordance with the histopathology-based diagnosis was performed. Major discordance was considered when it impacted management. **Results:** At ≥0.9 CS (n= 147, representing 61.25% of all samples), there were 116 (78.9%) concordant cases. At a CS of >=0.9 there were 82 (55.8%) gliomas, 66 of which were concordant (80.5%). There were 19 (12.9%) meningioma, 16 of which were concordant (84.2%), 18 (12.2%) cases of embryonal tumors, 16 of which were concordant (88.9%), 10 (6.8%) glioneuronal tumors, 6 of which were concordant (60%), and 8 (5.4%) ependymomas, all of which were concordant (100%). Among the discordant cases, there were 2 recent entities, including the newly described glioneuronal tumor with ATRX-alteration, kinase-fusion and anaplastic features (one case), and PLAG amplified tumor (one case). Interestingly, there were 6 (4.0%) high grade glioma samples at a CS >0.9 that were misclassified as inflammation/ normal tissue by the classifier. **Conclusion:** Our initial experience shows a good concordance rate between the initial histopathology-based diagnosis and the methylation profiling. DNA methylation profiling is a strong tool that helps in refining the diagnosis of CNS tumors, which might impact management. **Keywords:** CNS tumors – DNA methylation profiling – molecular classification

P093

Dynamic m6A RNA modification as a driver of cellular plasticity in glioma

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Introduction: Despite aggressive treatment, glioblastoma patients suffer from an invariably poor prognosis. Whilst substantial progress has been made in understanding the role of DNA methylation in this pathology, methylation of RNA has received comparatively little attention. N6-methyladenosine (m6A) is the most abundant RNA modification and is, crucially, reversible. The immense heterogeneity and plasticity of glioblastoma are major barriers to treatment, and dynamic m6A methylation may mediate such behavior, by flexibly influencing transcriptional networks via the regulation of mRNA metabolism. Objectives: We sought to characterize m6A modifications within their sequence context, to better understand biological pathways implicated by such modifications. **Methods:** Nanopore sequencing technology uniquely enables direct sequencing of RNA, and detection of RNA modifications within their sequence context. We present sequencing data from four in-house glioma-derived cell lines. We validate this approach using a novel drug inhibitor of the major m6A methyltransferase, METTL3. **Results:** We define transcriptome-wide RNA methylome profiles and identify common candidate pathways which exhibit methylation. We confirm robust reduction in m6A following treatment with a novel METTL3 inhibitor. We compare the transcriptional landscape of wild type tumors against these treated samples, demonstrating differential expression of biological processes linked to RNA metabolism. **Conclusion:** We demonstrate that nanopore direct RNA sequencing can resolve m6A modifications at single-base resolution. We validate this approach with a known inhibitor of METTL3 and further identify differentially expressed pathways. We show that glioma cell lines demonstrate extensive methylation throughout the transcriptome and exhibit a degree of inter-tumoral heterogeneity of these m6A signatures. **Keywords:** RNA – nanopore – epitranscriptomics – m6A – plasticity

P094

Extensive clear cell change in high-grade astrocytoma with piloid features (HGAP): A unique phenotype not yet described

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Introduction: High-Grade Astrocytoma with Piloid Features (HGAP) is a recently described tumor entity defined by a distinct DNA methylation profile. It frequently arises in posterior fossa and exhibits nonspecific morphology, often mimicking glioblastoma or pleomorphic xanthoastrocytoma. This report presents an HGAP with extensive clear cell change, a histological feature not previously documented. **Clinical:** A 49-year-old male presented with ataxia and nystagmus secondary to a right cerebellar hematoma identified through brain tomography. Initial medical management resulted in partial recovery. However, six months later, the patient's ataxia worsened. MRI revealed a contrast-enhancing mass measuring 46 mm at the hematoma site. Subtotal tumor resection was performed. Histological study demonstrated glial proliferation with focal cerebellar infiltration and high-grade features, including necrosis and microvascular proliferation. The tumor exhibited a predominant clear cell pattern with a small piloid-like region and occasional eosinophilic granular bodies. Immunohistochemically, the tumor was positive for GFAP, OLIG2 and synaptophysin, and negative for IDH1, with loss of ATRX expression and retention of H3-K27me3. Molecular analysis discarded IDH and H3 mutations and identified CDKN2A/B deletion. DNA methylation profiling confirmed HGAP with a classifier score of 0.99. The patient underwent radiation therapy and adjuvant Temozolamide chemotherapy and remains in stable disease three years after diagnosis. **Conclusions:** A matching DNA methylation profile is necessary for HGAP diagnosis following WHO criteria. Even in the presence of unusual features such as clear cell change, HGAP should be considered in the differential diagnosis of IDH/H3wt glial tumors in the cerebellum, prompting an adequate molecular workflow. **Keywords:** High-grade astrocytoma with piloid features – clear cell glioma – oligodendroglioma-like – high-grade astrocytoma – DNA methylation – posterior fossa

P095

Genomic heterogeneity drives distinct infiltration patterns in glioblastoma

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Introduction: Glioblastoma is the most common and malignant type of primary brain cancer with a median patient survival of only 15 months. Tumors are typically categorized into three molecular subtypes—proneural, classical, and mesenchymal—each linked to specific genomic and microenvironmental factors. However, the functional and clinical implications of these subtypes remain poorly understood. Standard treatment involves surgical resection followed by chemotherapy and radiotherapy. Despite this, malignant cells have already at the time point of diagnosis infiltrated healthy brain tissue, driving tumor recurrence. Infiltrating cells have been found to maintain high proliferative rates while invading the brain (termed "go-and-grow") but have also been shown to be non-proliferative (termed "go-or-grow"). **Methods and objectives:** Here, we perform analyses on transcriptomic data from over 600 patients, supplemented with genomic data, to investigate infiltration patterns in glioblastoma tumors. **Results:** We identify a proliferative astrocytic state strongly associated with the classical subtype and EGFR amplification and distinct from a non-proliferative astrocytic state. Further analysis showed subtype-specific infiltrative patterns between the tumor core and adjacent brain tissue. To validate these findings, we performed single-cell spatial transcriptomics using the CosMx platform, using a customized and commercial panel of 990 genes on patient tumor samples. This showed classical tumors exhibit a "go-or-grow" phenotype, where core regions are proliferative and infiltrative regions are dormant. In contrast, proneural tumors maintain consistent malignant states and proliferation rates, reflecting a "go-and-grow" phenotype. **Conclusion:** Together, our study provides insights into genomically-driven infiltration patterns in proneural and classical glioblastomas, with potential implications for clinical treatment. **Keywords**: Glioblastoma – spatial transcriptomics – infiltration

P096

GFAP negative chordoid glioma: A report of two cases

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Introduction: Chordoid glioma is a rare, circumscribed glioma, typically arising in the anterior portion of the third ventricle. It is usually distinguished from chordoid meningioma by positive immunohistochemical staining against GFAP and TTF1. Molecularly it is characterized by a specific D463H missense mutation in the PRKCA gene, which has not been described in any other tumor type. **Patients & methods:** In this report we describe two tumors arising in a 42-year-old female and a 60-year-old male. Both tumors were located in a suprasellar location in the wall of the third ventricle. The tumors displayed chordoid morphology, with pushing borders. Both tumors were GFAP negative, EMA positive, TTF1 positive and brachyury negative, raising a differential of chordoid glioma vs. chordoid meningioma. In both cases we performed Sanger sequencing which detected a PRKCA D463H mutation, confirming the diagnosis of chordoid glioma. **Conclusion:** Due to their rarity chordoid gliomas are not as well characterized as more common glial neoplasms. This report adds to the characterization of chordoid gliomas and highlights that we should not be over reliant on single markers but have to carefully consider the differential possibilities in a morphological context with thoughtful use of molecular methods. **Keywords:** Chordoid glioma – GFAP – PRKCA

P097

High-grade glioma with pleomorphic and pseudopapillary features (HPAP): Case reports and literature review

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Introduction: HPAP is a newly identified methylation class in the NCI/Bethesda classifier, displaying histological features of PXA, astroblastoma, ependymoma, PLNTY, and glioblastoma. It frequently presents chromosome 13 loss and TP53, RB1, and NF1 mutations. **Clinical**: Case 1: A 22-year-old man with generalized tonic-clonic seizures. MRI showed a temporal, circumscribed solid-cystic tumor without contrast enhancement. Histology revealed a diffuse oligodendroglioma-like tumor with nuclear pseudo inclusion, focal necrosis, and microvascular proliferation. Tumor cells were GFAP, CD34, and Olig2 positive, IDH1 negative, with preserved ATRX. DNA/RNA sequencing identified an *FGFR2-CTNNA3* fusion. CNV analysis detected chromosome 13q monosomy. Case 2: A 58-year-old man with seizures and memory impairment. MRI showed a temporal, circumscribed solid-cystic tumor with calcifications and heterogeneous contrast enhancement. Histology revealed a circumscribed tumor with pseudo rosettes of cells with plump eosinophilic cytoplasm and nuclear pseudo inclusions. Calcifications, perivascular hyalinization, and focal necrosis were observed. Tumor cells were GFAP, Olig2, neurofilament, and CD34 positive, IDH1 negative, with preserved ATRX. A *BRAF* V600E mutation was found, without no *CDKN2A* deletion. Neither case matched any group in the Heidelberg classifier but was categorized as HPAP in the NCI/Bethesda classifier. **Conclusions**: HPAP is a new heterogenous entity, with only 32 cases reported. We described 2 additional cases: one with histological and molecular features previously associated with PLNTY but with chromosome 13q monosomy, and the other with glioneuronal features, pseudo rosettes, and a BRAF V600E mutation, without chromosome 13 loss. Both patients are alive without disease progression after treatment (follow-up: 19 months and 66 months, respectively). **Keywords**: HPAP – glioma – PXA – glioblastoma – PLNTY

P098/WS11

Histopathological and molecular characterization of pediatric-type diffuse high-grade gliomas, H3-wildtype and IDH-wildtype: An Italian multicenter study

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Introduction: Pediatric-type diffuse high-grade gliomas, H3-wildtype and IDH-wildtype (pHGGs, H3-/IDH-WT), represent a recently recognized entity in the 2021 CNS WHO classification. Histopathological and immunohistochemical features are diverse and underexplored in previous studies. DNA methylation profiling is often necessary for diagnosis and identifying molecular subtypes. This study presents an Italian multi-center experience, focusing on histopathological and molecular characterization of this novel entity. **Materials & methods:** 50 pHGGs, H3-/IDH-WT cases were analyzed. Data collected included clinical course, imaging findings, surgical details, histopathology, immunohistochemical profiles, molecular alterations, and DNA methylation analysis. **Results:** The patient age range was 1 to 26 years. Tumors were primarily located in the cerebral hemispheres (n=44), with others in the cerebellum (n=2), mesencephalon (n=1), thalamus (n=1), fourth ventricle (n=1), and spinal cord (n=1). On MRI, all lesions showed T1w hypo- to isointensity and T2w hyperintensity, with 32 cases exhibiting inhomogeneous contrast enhancement. Histologically, tumors displayed diverse morphologies, including embryonal-like, PXA-like, sarcoma-like, PA-like, and astroblastoma-like features. Immunohistochemically, all tumors were GFAP-positive, with partial Olig2 positivity. Synaptophysin expression was noted in some cases, particularly in embryonal-like areas. 6 tumors exhibited ATRX loss, while 3 showed CDKN2A/2B homozygous deletions, 4 had MYCN amplification, and 3 had MYC gains. All tumors classified in the DNA methylation class of diffuse pediatric-type high grade glioma, within specific subtypes (n=21, RTK1; n=12, RTK2, n=9, MYCN). **Conclusions:** This study offers comprehensive insights into the clinical, histological, immunohistochemical, and molecular characteristics of pHGGs, H3-/IDH-WT, contributing to a better understanding of their heterogeneity and aiding in diagnostic challenges. **Keywords:** Pediatric-type diffuse high-grade glioma – H3-wildtype – IDH-wildtype – histopathology – DNA methylation – molecular subtypes

P099

Immune infiltrate assessment in primary and recurrent GBM -- validation of a scoring system for standardized assessment

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Introduction: Significant heterogeneity concerning the quantity, localization and composition of immune cell infiltration within Glioblastoma, IDH-wild type (GBM) have been reported. Novel treatment targets for GBMs, especially immune therapy, are currently under development. This requires standardized assessment of the immune infiltrate to be able to draw conclusions regarding changes induced by these novel treatment approaches. **Objectives:** This study aims at the validation of the first standardized scoring system for the characterization and quantification of the immune

infiltrate in GBMs on formalin-fixed paraffin-embedded (FFPE) material using immunohistochemistry (IHC). **Materials & methods:** IHC staining of paired samples of resections of primary and recurrent GBMs in 20 adult patients receiving standard therapy (surgical resection followed by radio-chemotherapy) for CD3/CD8/CD45/CD68/PD-1 were performed. All 40 cases were scored twice by three pathologists to allow assessment of intra- and interobserver variability. Scoring of immune cells was performed for four regions separately: intratumoral,perivascular and parenchymatous cells; and for peritumoral perivascular and parenchymatous cells. **Results:** Intra- and interobserver variability for CD3/CD8/PD1 was minimal. CD68-positive cells were the most prevalent inflammatory cells in primary and recurrent tumors in all regions, especially in the intra-tumoral parenchyma. We did not observe significant changes between primary and recurrent tumors in the quantity of inflammatory cells; neither for the different immune cell subsets, nor for different localization of inflammatory cells. **Conclusion:** We propose a feasible, cost-efficient and robust strategy to assess the immune infiltrate on FFPE-material that allows for standardized comparison of inflammation between patients, with applications for ongoing clinical trials. **Keywords:** Glioblastoma – immune therapy – immune infiltrate – clinical trial – inflammation

P100

Immunohistochemical expression and differential methylation of HOXB13 reliably distinguishes myxopapillary ependymoma from spinal ependymoma

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Introduction: Histological distinction of spinal ependymoma and myxopapillary ependymoma may be difficult in individual cases and molecular diagnostic assessment, in particular DNA methylome profiling, may then be required to assign the correct diagnosis. Expression of the homeobox gene *HOXB13* at the mRNA and protein levels is frequent in myxopapillary ependymoma. **Objectives:** Here, we evaluated the diagnostic role of HOXB13 immunostaining in a large institutional cohort of spinal neoplasms, including different types of spinal ependymal tumors and other relevant differential diagnoses. **Materials & methods:** Expression of HOXB13 protein was compared to molecular findings obtained by bead array-based DNA methylation and DNA copy number profiling, as well as next generation gene panel sequencing. **Results:** Collectively, our findings support that strong nuclear HOXB13 immunopositivity is a reliable diagnostic marker for molecularly confirmed myxopapillary ependymoma. Using targeted DNA methylation analyses, we additionally show that differential HOXB13 protein expression is related to differential *HOXB13*-associated CpG site methylation in myxopapillary versus spinal ependymomas. **Conclusion:** Thus, immunohistochemistry for HOXB13 protein expression and/or targeted DNA methylation analysis of *HOXB13* may substitute for global DNA methylome profiling in routine diagnostics to facilitate precise classification of spinal ependymal tumors. **Keywords**: Immunohistochemistry – HOXB13 – myxopapillary ependymoma – spinal ependymoma – molecular diagnostics – targeted methylome analysis

P101

Improved and rapid CNS tumor diagnostics using Nanopore sequencing

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Introduction: DNA-methylation profiling is an important tool for CNS tumor classification, but it is time-consuming and expensive. Nanopore sequencing (NPS) is a rapid and cost-effective method, enabling a methylation-based diagnosis within hours. **Objectives:** We recently implemented NPS in the routine diagnostics of CNS tumors, and here we report on our experience. **Materials & methods:** 192 CNS tumor samples were analyzed from August to December 2024. Sections for DNA extraction were prepared in parallel with frozen sections. Low-pass whole genome sequencing was performed using a GridION (Oxford Nanopore Technologies). Data was analyzed using the nanoDx pipeline (by Philipp Euskirchen) to obtain DNA methylation-based classification and copy number variations (CNVs). NPS was mainly performed when a glioma, meningioma or embryonal tumor was suspected. **Results:** In 89% (49/55) of IDH-wildtype GBMs the NPS-based methylation class matched the final diagnosis (calibrated score ≥0.2). In all gliomas (24/24) classified as IDH-mutant, the IDH mutation was confirmed. For meningiomas, 100% (60/60) matched the final diagnosis. In 28% (17/60) of meningiomas 1p/22q-loss were detected, confirming them to be at least WHO grade 2. Three of five ependymomas and two of two embryonal tumors were correctly classified with specific subtypes. **Conclusion:** NPS allows rapid and robust methylation-based classification in gliomas, meningiomas, and pediatric CNS tumors. It provides accurate molecular diagnoses within 1-2 days, reducing the number of IHC-stainings and other molecular analyses. It also identifies CNVs useful for grading and classification, as well as clinically relevant subgroups in pediatric CNS tumors. **Keywords:** Glioma – meningioma – nanopore sequencing – DNA-methylation – classification

P102

Infant type hemispheric gliomas: rare tumors with emerging molecular and clinical insights

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Introduction: Although brain tumors are among the most common neoplastic diseases in early pediatric age, they are still considered rare. While substantial literature exists on embryonal tumors like medulloblastomas, infant type hemispheric gliomas (IHGs) with receptor tyrosine kinase (RTK) fusions remain understudied. Notably, these tumors lack an assigned WHO grade, underscoring the gaps in knowledge. **Objectives:** To determine the incidence and the clinical features of IHGs diagnosed within the first three years of life. **Materials & methods:** This study analyzed data from eight institutions in Turkey. Intracranial tumors diagnosed in children aged 0–36 months were categorized into high-grade diffuse glial tumors (excluding H3-mutant), embryonal tumors, ependymal tumors, other gliomas (e.g., circumscribed gliomas, H3-mutant gliomas), and other tumors. Clinical and demographic data were collected. **Results:** Among 462 cases, 19 were high-grade diffuse gliomas, 146 embryonal tumors, 70 ependymomas, 155 other gliomas, and 72 other tumors. Molecular analysis of 5 high-grade gliomas revealed *ALK* fusions in 2 cases, and *MET, NTRK2*, and *NTRK3* fusions in 1 case each. Three patients died, with a mean follow-up of 40.43 months (range: 18 days–172.5 months). Two cases had follow-up shorter than one month. Survival rates were 60.0% at 1 year, 45.0% at 2 years, and 20.0% at 5 years. **Conclusion:** Despite their high-grade classification, IHGs show variable outcomes. This study emphasizes the need to investigate survival predictors and the role of RTK fusions in these rare tumors. **Keywords:** Infant type hemispheric glioma – receptor tyrosine kinase – infant – glial tumor – glioma

P103

Longstanding posterior fossa subependymomas with sarcomatous differentiation harbor concomitant TERT promoter and TP53 mutations - report of two cases

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Background: Subependymomas are generally benign, slow-growing tumors classified as CNS WHO grade 1. Malignant transformation of subependymomas is extremely rare and has not been extensively studied at the molecular level. Here, we report on the epigenetic and genetic profiles of two cases. **Case reports:** A 59-year-old male (Case 1) presented with a two-year history of progressive weight loss, dizziness, double vision, and vomiting due to a heterogeneously enhancing lesion arising from the floor of the fourth ventricle. Case 2 (62-year-old man) initially presented in 2016 with a small fourth ventricular lesion on imaging and has recently undergone tumor resection due to rapid deterioration. Histology of both cases revealed rather heterogeneous tumors with an apparent GFAP and EMA-positive ependymal component and extensive sarcomatous differentiation, including bizarre multinucleate and spindle-shaped cells as well as myoid and osteoid differentiation. Methylation profiling (DKFZ, brain classifier v12.8) of the ependymal component indicated posterior fossa subependymoma in both cases with loss of chromosome 6. Case 2 also harbored multiple gene amplifications *(PDGFRA, MAF* and *CCNE1)*. In contrast, the sarcomatous area was profiled as "undifferentiated sarcoma" (DKFZ, sarcoma classifier v12.2) with multiple CNV alterations. Targeted next generation sequencing identified pathogenic *TERT* promoter and *TP53* variants in both components. An in-frame *MAML2::PPP2R1B* duplication was found in Case 2. Both patients passed

away due to tumor progression (OS: 1 and 3 months). **Conclusion:** Our results suggest that longstanding posterior fossa subependymomas may undergo sarcomatous transformation through acquisition of concomitant *TERT* promoter and *TP53* mutations. Early surgical resection may be considered. **Keywords:** Subependymoma – sarcomatous transformation – DNA methylation profiling – TERT promoter mutation – TP53 mutation – copy number variation

P104

Mapping the rules of glioblastoma with integrated single cell and spatial genomics

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Introduction: The malignant brain tumor glioblastoma multiforme (GBM) displays significant levels of cellular heterogeneity and plasticity. However, we lack fundamental insight into how diverse malignant cell states arise and organize in GBM. **Objectives:** We developed GBM-space, a new collaborative effort aiming to map the cellular and tissue architecture of GBM using multi-modal transcriptomics. **Patients and methods:** We deeply profiled 12 distinct IDH-wild type tumors across multiple sites. For each patient and at each tumor site we generated both sequencing and imaging-based spatial transcriptomics. **Results:** Integrating sequencing and imaging-based spatial transcriptomics, we discovered that malignant cell states regionally segregate into distinct spatial tissue niches in GBM. We found that these niches recur across patients, reflecting a shared tissue organization across GBM. Such spatial niches defined by spatial transcriptomics closely map onto well-established histologically defined tumor regions. Furthermore, we find that that the spatial organization of malignant cells reflects their stereotyped cellular trajectories from neurodevelopmental-like to injury response and hypoxia states during tumor expansion. **Conclusions:** Our efforts reveal novel spatial organization of malignant cell states in glioblastoma across a dominant cellular trajectory. **Keywords:** Glioblastoma multiforme – spatial transcriptomics – single cell transcriptomics

P105

MTAP and p16 as immunohistochemical surrogates of CDKN2A/B homozygous deletion in central nervous system tumors: A multicentre Italian experience

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Introduction: Molecular tests including gene sequencing or fluorescence in situ hybridization (FISH) have been traditionally used to assess CDKN2A/B homozygous deletion (HD) but the use of immunohistochemical surrogates such as MTAP and p16 is gradually emerging due to lower costs and relative widespread availability. **Objectives:** We studied the concordance rate between immunohistochemistry for MTAP and p16, and CDKN2A/B status assessed by FISH and further evaluated the correlation between these markers and survival on a multicenter series of CNS tumors. **Materials & methods**: Our series included 227 patients, diagnosed with different tumor types, with glioblastoma IDH-wild type being the most prevalent (n = 64; 28.2%), followed by meningioma (n = 61; 26.9%), IDH-mutant astrocytoma (n = 52; 22.9%), IDH-mutant and 1p/19q codeleted oligodendroglioma (n = 35; 15.4%), and pleomorphic xanthoastrocytoma (n = 15; 6.6%). **Results:** In all tumor types, most cases with CDKN2A HD exhibited MTAP loss and p16 negativity (p-values<0.05). The combined diagnostic utility of MTAP and p16 in identifying CDKN2A status yielded a sensitivity of 92%, specificity of 80%, positive predictive value of 86%, and negative predictive value of 88%, suggesting that integrating MTAP and p16 statuses does not enhance the diagnostic accuracy achieved by MTAP alone. The survival analysis revealed significantly lower disease-free survival and overall survival among patients with MTAP loss, p16 negativity, and CDKN2A HD. **Conclusion:** Immunohistochemistry for MTAP with or without p16 association may be confidently used as surrogate of CDKN2A/B HD in CNS tumors due to its lower cost and rapid feasibility. **Keywords:** MTAP – p16 – brain tumor – CDKN2A/B – immunohistochemistry

P106

MTAP immunohistochemistry as a surrogate marker for CDKN2A homozygous deletion in a Belgian CNS tumor cohort

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Introduction: CDKN2A homozygous deletion (HD) is associated with poor prognosis in IDH-wild type and IDH-mutant gliomas and can be detected with different molecular methods (fluorescence in situ hybridization (FISH), NGS-based copy number variation (CNV), array-based whole genome methylation and others). Methylthioadenosine phosphorylase (MTAP) immunohistochemistry (IHC) has been proposed as a cost-effective and rapid alternative for molecular analysis. **Objectives:** We present a validation study of a Belgian cohort of diffuse IDH-wild type and IDH-mutated glioma, in which MTAP IHC was compared to CDKN2A HD. **Materials & methods:** All diffuse gliomas (IDH-mutant or IDH-wild type) from 1/01/2024 to 31/12/2024 with available MTAP IHC and a CDKN2A molecular result were included. The correlation between MTAP expression and CDKN2A HD was determined. Cases with a discrepant IHC and molecular results were analyzed in more detail. R**esults:** 73 glioma cases were included. 50 cases showed retained MTAP IHC (68.5%), confirmed by molecular analysis in 42 of 50 cases. 23 of 73 cases showed loss of MTAP IHC (31%), in 22 cases confirmed by molecular analysis. (PPV 96%, NPV 84%) 9 cases showed a discrepancy. Possible causes included low tumor cellularity and presence of necrosis. Additionally, we describe patient and histological characteristics. **Conclusion:** In this retrospective validation study, we confirmed that loss of MTAP protein expression can be used as a robust surrogate marker for CDKN2A homozygous deletion in glioma. We suggest caveats for

discrepancy and propose additional value of MTAP IHC in cases in which molecular analysis is not successful. **Keywords:** CDKN2A deletion – MTAP – glioma – immunohistochemistry

P107

Optimal qMSP cutoff for MGMT promoter methylation in glioblastoma: A clinically robust and longitudinally tracked patient series

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Introduction: Glioblastoma (GBM) is one of the most aggressive primary central nervous system tumors. O6-Methylguanine-DNA Methyltransferase (MGMT) promoter methylation is a critical biomarker influencing treatment response to alkylating chemotherapy. This study aims to determine an optimal quantitative methylation-specific PCR (qMSP) cutoff for MGMT promoter methylation and evaluate its prognostic significance across surgical subgroups and anatomical tumor locations. **Methods:** A retrospective study was conducted on 101 glioblastoma IDH-wildtype patients diagnosed between 2008 and 2022. MGMT methylation status was assessed via qMSP, with an optimal cutoff value determined using receiver operating characteristic (ROC) curve analysis. The clinical significance of this cutoff was validated by examining its association with surgical approach, anatomical tumor involvement, and overall survival (OS). Statistical analyses included Kaplan-Meier survival curves and Cox proportional hazards regression. **Results:** The optimal qMSP cutoff for MGMT promoter methylation was identified as 0.242. Patients with qMSP values above this threshold exhibited significantly prolonged survival compared to those below (median OS: 32 vs. 13 months, $p < 0.0001$). The survival benefit was particularly pronounced in patients undergoing gross total resection (median OS: 39 vs. 16 months, $p < 0.05$) and those with neocortical involvement (median OS: 23.8 vs. 8.6 months, $p = 0.0218$). Furthermore, MGMT methylation demonstrated prognostic significance in white matter fiber involvement, with high qMSP values correlating with improved survival in cases involving long association fibers, commissural bodies, and the subventricular zone ($p < 0.01$). Conversely, qMSP status did not significantly impact survival in projection tract involvement ($p = 0.5350$). Additionally, patients with multifocal tumors and those undergoing stereotactic biopsy had worse outcomes, reinforcing the importance of resection when feasible. **Conclusion:** This study establishes a clinically relevant MGMT promoter methylation cutoff for qMSP (0.242) and highlights its impact on surgical and anatomical prognostic factors in glioblastoma. The findings emphasize the prognostic value of MGMT methylation in patient stratification and treatment planning. **Keywords:** Glioblastoma – MGMT – qMSP

P108

Pleomorphic xanthoastrocytoma: Integrating morphological, immunohistochemical and molecular profiles in the diagnosis

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Introduction: Pleomorphic xanthoastrocytoma (PXA), a rare, circumscribed astrocytoma, affecting predominantly children and young adults, is characterized by a distinctive morphology, MAPK-pathway alterations, most frequently BRAFV600E mutation and CDKN2A/2B homozygous deletion. A distinct DNA PXA methylome profile has also been reported. **Objectives:** To assess the role of morphology, immunohistochemistry (IHC) and molecular findings, including methylome profiling in the diagnosis of PXA. **Methods:** Upon review of archived diagnostic slides, 12 histologically confirmed PXAs (9 grade 2, 4 grade 3) and 1 tumour with borderline morphology between pilocytic astrocytoma and PXA, all BRAFV600E mutant by IHC and/or sequencing, underwent p16 and MTAP IHC and Methylation EPICv2 (Illumina) array. The idat files were analyzed through the DKFZ(v12.8) and the NCI-Bethesda(v2.0) classifiers. **Results:** 12 cases lacked p16 expression, 10 (83%) with concomitant MTAP-loss consistent with CDKN2A biallelic inactivation and supportive of the diagnosis, while the borderline case only showed MTAP-loss. Respectively 8 and 9 (of 12) cases matched to methylation class (mc) PXA with a calibrated score > 0.9 with the DKFZ (67%) and NIH-Bethesda classifiers (75%). Discordant cases had a low score for mcPXA (2 in DKFZ), suggested other mc (1 in NIH-Bethesda) or were no match (2 in each classifier). The borderline case matched to mcPXA only in the NIH-Bethesda classifier. All 13 cases clustered with PXA in the Brain-UMAP. **Conclusions:** Morphology remains a robust predictor of PXA diagnosis. IHC including BRAFV600E, p16, MTAP is highly helpful in supporting the diagnosis. DNA methylome profile is primarily confirmatory and may be helpful in cases with borderline morphology.
Keywords: Pleomorphic xanthoastrocytoma – CDKN2A – p16 – MTAP – methylome

P109

Polymorphous low-grade neuroepithelial tumor of the young harboring FGFR3::TACC3 fusion with atypical aggressive clinical progression

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Background: Polymorphous low-grade neuroepithelial tumor of the young (PLNTY) is a rare, WHO grade 1 glial tumor, characterized by oligodendrogliocyte-like pathology, extravascular CD34 expression, calcifications and MAPK pathway alterations such as FGFR3::TACC3 fusions. **Clinical case:** A 46-year-old male presented with a 6-month history of focal seizures. Brain MRI showed a 29-mm

right temporal subcortical lesion with cystic and calcified components and ill-defined borders, with no mass effect or contrast enhancement, indicating a low-grade glial/glioneuronal tumor. Over six months, the lesion grew and showed slight contrast enhancement, leading to partial surgical resection. Histopathology revealed a moderately infiltrative glial tumor with an oligodendrogliocyte-like component and a more hypercellular component, with bland oval nuclei, arranged in fascicular pattern, rare mitoses, abundant calcifications, and a branched capillary network. Immunostaining showed GFAP and OLIG2 expression, extensive extravascular CD34 positivity, negative IDH1-R132H and BRAFV600E, retained ATRX, wildtype p53 expression and low proliferation index (ki67). Molecular testing revealed a FGFR3(18)::TACC3(11) fusion and TERT promoter mutation, fulfilling diagnostic criteria for PLNTY. Follow-up MRI after 12 months showed significant tumor growth, prompting re-intervention. Histopathology revealed the same morphological pattern but increased cellularity and mitoses, in the absence of necrosis or microvascular proliferation. Methylation profiling is pending. **Conclusion:** The FGFR3::TACC3 fusion is also found in a subset of glioblastomas and low-grade glial tumors with recurrent morphological features (oligodendrogliocyte-like pathology, extravascular CD34, calcifications) that overlap with PLNTY. Our case displayed an atypically aggressive behavior for PLNTY, questioning this diagnosis. Further studies are needed to clarify whether this tumor represents a malignant spectrum or a distinct entity. **Keywords:** Polymorphous low-grade neuroepithelial tumor of the young (PLNTY) – FGFR3::TACC3 fusion – glioma – oligodendrogliocyte-like pathology

P110

POU2F3 in adult-type diffuse gliomas: A new prognostic biomarker?

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Introduction: Adult-type diffuse gliomas are central nervous system tumors with high heterogeneity and variable prognosis. POU2F3 is a transcription factor involved in cellular differentiation and has been shown to have prognostic significance in various cancer types. Objectives: This study aims to investigate the potential prognostic value of POU2F3 in adult-type diffuse gliomas. **Materials & methods:** Between January 2021 and January 2024, POU2F3 expression was analyzed using immunohistochemical methods in adult-type diffuse gliomas diagnosed, treated, and followed up at Sivas Cumhuriyet University Faculty of Medicine Research and Education Hospital. **Results:** The median age of the 40 patients included in the study was 61 (range 29-76) years, Among the patients, 8 (20%) had an IDH mutation, 10 (25%) had ATRX loss, 15 (37.5%) had a Ki67 proliferation index greater than 50%, and 23 (57.5%) had a p53 mutation. POU2F3 expression was observed as nuclear staining in tumor cells, while no expression was detected in glial tissue, with this difference being statistically significant ($p<0.001$). POU2F3 expression was detected in 8 patients (20%). POU2F3 expression was observed to be positive in IDH wild-type, ATRX loss-negative, p53 mutant, and Ki67 index <50 cases, but these relationships were not statistically significant ($p>0.05$). The median overall survival was 11 months (95% CI: 4.8-17.1) for patients without POU2F3 expression and 5 months (95% CI: 1.0-10.5) for those with POU2F3 expression, with no statistically significant difference observed between the groups. ($p=0.399$). **Conclusion:** POU2F3 expression was detected in adult-type diffuse gliomas, but no significant prognostic relationship was found with clinical data and survival. **Keywords:** Diffuse gliomas – POU2F3 – immunohistochemistry – prognosis

P111

Prognostic value of CIC and p16 in oligodendroglioma

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Introduction: Oligodendrogliomas (ODG) constitute 3.5% of primary brain tumors. Although there have been great advances for the diagnosis of adult type glial tumors since WHO 2016 classification, indicators for predicting survival are still being investigated. **Objective:** Aimed to investigate the prognostic significance of CIC and p16 expression in ODG. **Methods:** Ninety-two cases diagnosed with ODG, IDH mutant and 1p 19q co-deleted in our department between 2016 and 2022 were selected. CIC (Invitrogen, PA1-46018, 1/100, 45 minutes) and p16 (Bio SB, RM267, ready to use, 45 minutes) antibodies were applied to all cases by immunohistochemical method with an autostainer (Leica Bond Max). Cases were divided into 2 groups according to expression rates: ≥1% and 0%. The results were compared with tumor grade, Ki 67 index and CNVs. Kaplan-Meier curve was used for disease-free survival (DFS) and overall survival (OS) analysis. **Results:** Mean age was 40,1 (14-73), and M/F was 1,9 . Mean follow up time was 65,8 months (range: 3-285 months). Grade 2/grade 3 ratio was 51/41. Cases with ≥6 mitosis/10HPF, Ki 67 index ≥15% , were independently associated with shorter DFS (p=0,0002 and p=0,0006; respectively), but not OS. CIC expression loss was higher in grade 3 tumors and associated with shorter DFS (p=0,008 and p=0,04; respectively) but not OS. P16 loss was not associated with survival. In multivariate analyses, together CIC and p16 expression loss was associated with higher tumor grade. **Conclusion:** Although molecular tests are required for definitive results, CIC IHC may be used as a screening biomarker. **Keywords**: Oligodendroglioma – CIC – p16 – survival

P112

PTEN homozygous deletion is a negative prognostic factor in TTFields-treated glioblastoma, IDH wildtype

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Introduction: Tumor Treating Fields (TTFields) are an adjunctive treatment for glioblastoma, isocitrate dehydrogenase wildtype (IDH wt). TTFields indication is not standardized; patient selection is mostly based on a high KPS. **Objective:** This study aims to identify molecular biomarkers guiding clinical decisions on TTFields initiation. **Patients & methods:** A retrospective analysis was conducted on 64 patients with primary glioblastoma, IDH wt treated with surgery, radiochemotherapy, followed by TTFields. Clinical data were collected. Tumors underwent TSO500 DNA/RNA sequencing and methylation profiling (EPIC). Alterations present in ≥5 patients were analyzed for association with overall survival using Kaplan-Meier and Cox models. Methylation class, TMB, and signaling pathway

activation were also assessed for survival. Two TTFields-naïve glioblastoma, IDH wt cohorts served as controls (combined n=175). **Results:** Molecular profiling revealed 25 alterations (e.g., mutations: PTEN, EGFR, deletions: PTEN, CDKN2A/B; amplifications: EGFR). Univariate analyses showed preoperative KPS and MGMT promoter methylation as protective, while EGFR amplification, CDKN2A/B, and PTEN homozygous deletions were linked to worse survival. Multivariate analysis confirmed KPS and MGMT as protective and PTEN homozygous deletion as a significant risk factor (HR: 3.86, 95% CI: 1.51–9.87, p=0.0049). Comparative analysis with TTFields-naïve cohorts showed no link between PTEN homozygous deletion and worse outcomes, with deletion rates comparable across cohorts (controls: 7%, TTFields: 11%). **Conclusion:** Besides established protective outcome factors MGMT and KPS, in our cohort of glioblastoma, IDH wt patients treated with TTFields, PTEN homozygous deletion was significantly associated with worse survival. PTEN deletion status may predict reduced benefit from TTFields, warranting testing before treatment initiation. **Keywords**: Glioblastoma isocitrate dehydrogenase wildtype – tumor treating fields – molecular profiling – PTEN homozygous deletion – overall survival

P113

RB1 and TP53 pathway signaling alterations contribute to the development of the primitive neuronal component in glioblastoma

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Introduction: Glioblastoma with Primitive Neuronal Component (GBM-PNC) represents a GBM variant characterized by conventional GBM areas along with immature early neuronal components. We recently reported that RB1 and TP53 pathway alterations represents GBM-PNC molecular hallmarks and PNC phenotype relies on expression of EBF3, an early neurogenic transcription factor, directly controlled by MYC transcription factors in accessible chromatin sites. **Objectives:** We established in vitro models to test if p53 and RB1 pathway signaling impairment is required to commit conventional GBMs toward a GBM-PNC phenotype. **Materials & methods:** We selected RB1-wildtype patient-derived glioma stem cells and created a CRISPR/Cas9 RB1 knockout model with either loss or retention of p53 functionality. RB1-mutated GBM-PNC GSC line was transduced with a lentiviral vector to test if RB1 ectopic expression could revert the GBM-PNC phenotype. **Results:** Interestingly, cell cycle assays showed an increasing polyploidy in TP53-RB1 double mutant clones, reminiscent of mitotic aberrations typical of GBM-PNC. Double mutant TP53-RB1 organoids significantly downregulated expression of glial markers and showed persistent CD133 expression, suggesting an immature phenotype. Unexpectedly, we didn't observe EBF3 increased levels in double mutant clones, suggesting a possible underlying epigenetic status that might prevent, or facilitate, the development of a full GBM-PNC phenotype. Unsuccessful ectopic expression of RB1 in GBM-PNC GSCs reinforced this hypothesis. **Conclusions:** RB1 loss alone is not sufficient to trigger the switch from conventional GBM into GBM-PNC. Thus, concomitant p53 and RB1 pathways alterations represent a predisposing feature, and

additional epigenetic status will be necessary to obtain a full GBM-PNC phenotype. **Keywords:** Glioblastoma with primitive neuronal component – RB1 – TP53 – CRISPR/Cas9 – MYC

P114

Role of 10q loss, CDKN2A deletions, and EGFR amplification in the survival of IDH-mutant astrocytomas

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Introduction: According to the WHO 2021 classification, the only molecular parameter that affects the grading criteria for IDH-mutant astrocytoma (IDHmut-A) is the *CDKN2A/B* homozygous deletion. As tumors progress from grade 2 to grade 4, it is crucial to identify factors that influence overall survival (OS). **Objectives**: To evaluate the impact of molecular alterations traditionally associated with high-grade gliomas within the grading scheme for IDHmut-A. **Materials & methods**: We retrospectively analyzed the role of 10q loss, *CDKN2A* deletions, and *EGFR* amplification in 189 IDHmut-A cases, reclassified according to the WHO 2021 criteria (grade 2, n=133; grade 3, n=18; grade 4, n=38). **Results**: Among the 189 cases, 29 presented with *CDKN2A* hemizygous deletion, 17 with *CDKN2A* homozygous deletion, and 20 showed *EGFR* amplification (18 of which were *EGFR* trisomy). Both multivariate and univariate analyses revealed that WHO grade, *EGFR* trisomy, and *CDKN2A* homozygous deletion significantly impacted OS. However, *CDKN2A* hemizygous deletion and 10q loss did not influence OS in our cohort. Given that 11 of the 18 cases with *EGFR* trisomy were grade 2 IDHmut-A, we compared grade 2 tumors with and without *EGFR* trisomy and found worse OS in the trisomy cases (*p=0.0034*). **Conclusions**: Our results suggest that *EGFR* trisomy plays a significant role in the survival of IDHmut-A, similar to grade 4, and could be considered an additional molecular criterion for high-grade IDHmut-A. Further research is needed to determine whether *EGFR* trisomy's impact is an independent marker or if it is linked to other molecular alterations that affect OS. **Keywords:** Astrocytoma – IDH – EGFR – CDKN2A – 10q

P115

Ruta graveolens water extract affects GBM migration, invasion and vasculogenic mimicry but exerts a low toxicity profile on primary brain cells

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Introduction: Glioblastoma (GBM), the most aggressive and highly vascularized brain tumor, during tumor progression, may undergo the process of vasculogenic mimicry (VM), further promoting tumor

malignancy. GBM treatments are mostly based on a combined surgical, radio- and chemotherapy. Chemotherapeutic agents, including temozolomide (TMZ) are known to damage differentiated healthy brain cells and cause cognitive impairment often referred to “chemo-brain”. The side-effects of this type of chemotherapeutic drugs are caused by the non-selective toxic action on healthy cells. **Objectives:** Herein, we analyze the effects of the natural compound Ruta graveolens water extract (RGWE) compared to Temozolomide, on GBM cell lines and on mouse primary brain cell cultures. **Methods:** Several viability assays were performed to test RGWE/TMZ effects on GBM cell lines and primary brain cells. Tube formation on geltrex was monitored and vessel-like structures formation was analyzed by PAS staining and VE-cadherin immunocytochemistry. Random and directional migration/invasion were evaluated by means of wound healing and Boyden chamber assays. Moreover, immunocytochemistry assays were performed to analyze neuronal markers. Finally, neurites analysis was performed using the ImageXpress Pico Machine. **Results:** RGWE inhibits migration, invasion and VM of GBM cell lines at sublethal doses. Furthermore, RGWE do not display toxicity on differentiated neurons, astrocytes, oligodendrocytes and microglia. Interestingly, in case of microglia it is able to promote its proliferation and activation. Moreover, RGWE appears more selective than TMZ to discriminate between tumor cells and healthy primary neuronal cells. **Conclusions:** Thus, RGWE might be a promising candidate to inhibit glioblastoma vascularization and cell viability with a low toxicity profile on normal brain cells. **Keywords:** Glioblastoma – natural compounds – primary brain cells – neurons – temozolomide – vasculogenic mimicry

P116

Spatially distinct patient-derived organoids as a model for intratumoral heterogeneity in glioblastoma

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Introduction: Glioblastoma (GBM) is the most common malignant primary brain tumor in adults characterized by pronounced intratumoral heterogeneity, which drives therapy resistance and tumor recurrence. **Objectives:** This study evaluates separately generated patient-derived 3D organoids (PDOs) retrieved from different tumor locations as potential model for capturing GBM heterogeneity. **Materials & methods:** To assess tumor heterogeneity, tissue samples were collected from six distinct regions of each GBM during tumor resection (n=10). Half of each sample was used for PDO generation, while the other half was utilized for characterizing the corresponding primary tissue (PT). Comparative analyses included high-resolution DNA methylation profiling using the Infinium MethylationEPIC v2.0 BeadChip. Data were explored using the Integrative Genomics Viewer. Statistical analyses were performed using R. Immunohistochemical staining of PT and PDOs will be performed to evaluate heterogeneity in protein expression. **Results:** Methylation analysis of all 10 patients was performed, with 3 to 6 PT samples and corresponding organoids analyzed per patient. PDOs methylation profiles accurately replicate the methylation of the corresponding PT. Significant differences were observed between samples from distinct regions. For instance, in one GBM, a CDK4 was strongly amplified in only three of four locations, and MDM2 was amplified in only one of four locations. **Conclusion:** The findings indicate that PDOs derived from different tumor regions reliably replicate the heterogeneity of PTs. PTs and PDOs exhibit intratumoral differences depending on the region of origin. Using PDOs

as an *ex vivo* model reflecting GBM's intratumor heterogeneity, enables the development of comprehensive drug testing panels for personalized therapy. **Keywords**: Glioblastoma – intratumoral heterogeneity – organoids – methylation analysis

P117

Sublethal doses of Temozolomide inhibit migration and vasculogenic mimicry of glioblastoma cells

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Introduction: Glioblastoma multiforme (GBM), the most devastating and widespread primary CNS tumor, is sustained by a remarkable vascularization occurring through different mechanisms, i.e. vasculogenic mimicry (VM), the non-endothelial formation of new vessels. Temozolomide (TMZ) is the gold standard treatments for gliomas, nevertheless chemoresistance and tumor recurrence are still unsolved issues. **Objectives:** Herein, we tested the effect of sublethal doses of TMZ on GBM cell lines, also at short time, in order to reduce side effects related to high dosages and to find new mechanisms of action exerted by TMZ instead of well-known alkylating ones. **Methods:** Lethal and sublethal doses of TMZ were identified by Trypan blue exclusion test. To test the effect of TMZ on VM, tube formation on a gelled matrix was monitored, while random and directional migration were evaluated by wound healing and transwell migration assays, respectively. Furthermore, phalloidin staining was performed to analyze effects exerted on cytoskeleton. Finally, immunocytochemistry analysis, with both neural and stemness markers, were performed. **Results:** Results showed that TMZ was able to reduce GBM migration, invasion and VM at sublethal doses. Moreover, it was able to reduce cytoskeleton reorganization at short time and to reduce stemness markers fluorescence. Interestingly, preliminary data showed that cisplatin, a commonly used chemotherapeutic agent for treating GBM, was ineffective in inhibiting VM and migration. **Conclusions:** In conclusion, these results indicate that sublethal doses of TMZ can still exert therapeutic effects on aggressive properties of GBM cells such as migration and VM. Thus, new mechanism(s) of action of TMZ are likely to be involved. **Keywords:** Glioblastoma – temozolomide – sublethal doses – stemness – vasculogenic mimicry – cell migration

P118

Supratentorial, extra ventricular ependymoma presenting as pediatric-type diffuse low-grade glioma

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Introduction: The 2021 WHO classification divides diffuse gliomas in adult and pediatric-types, the latter involving a large array of genetic alterations. Supratentorial ependymomas involve ZFTA or YAP1 gene fusions, are more common in children and often show no obvious ventricular connection. **Clinical:** We present the case of a 13 year old girl, diagnosed with a subcortical tumor, after epileptic seizures. A complete resection was performed in September 2022. Tumor morphology revealed a diffuse growth pattern with monomorphic oligodendroglial-like cells, low cellularity and no significant mitotic activity. While GFAP-positive, Olig 2 expression was largely absent. P 53 expression was slightly elevated but not significant while ATRX expression was retained and IDH 1 was negative. The lack of Olig 2 prompted the search for MYB/MYBL1 alterations. FISH analyses revealed no MYB or FGFR2 mutations but were inconclusive for MYBL1 so the diagnoses of pediatric-type diffuse low grade glioma NOS was maintained. 2 years later the tumor recurred, and a new resection was performed. This time, morphology had changed. Tumor cells showed increased mitotic activity and a rosetted and ribbon-like architecture. Olig 2 was again negative with faint, focal GFAP and strong membranous D2-40 and EMA. Astroblastoma was suspected due to similarities to a recent case. NGS sequencing only revealed TP53 and KRAS mutations, so methylation was performed, revealing a supratentorial ZFTA fusion ependymoma, grade 3. **Conclusion:** Unlike adult gliomas, pediatric tumors involve very diverse genetic alterations and significant morphologically overlapping features. Access to modern diagnostic tools is important to providing adequate treatment. **Keywords**: Pediatric glioma – low grade glioma – ependymoma – extra ventricular – supratentorial ependymoma – ZFTA fusion – diffuse glioma

P119

The diagnostic and clinical challenges associated with infant gliomas with RAF1 fusions: two case reports

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Introduction: High-grade gliomas (HGG) occur in adults and children and have a poor prognosis. Infant gliomas have been shown to encompass multiple subgroups characterized by specific molecular features; these include the infant-type hemispheric glioma (IHG) characterized by RTK fusions (NTRK, ROS1, MET, ALK), the desmoplastic infantile astrocytoma/ganglioglioma (DIA/DIG) (MAPK pathway alterations) and other pediatric-type gliomas. RAF1 fusions are a rare molecular feature of some low-grade gliomas. We present two cases of RAF1 fusion-positive infant gliomas, which classified as IHGs by methylation profiling. **Clinical cases:** The first case was a male infant (aged <1 year) presenting with seizures. An MRI scan identified a hemispheric lesion with evidence of spinal metastasis. A resection of the temporal tumor was undertaken. The second case was a male infant (aged <1 year) who presented with focal seizures. A right hemispheric tumor was resected. The histology of both tumors showed a glial/glioneuronal tumor within the spectrum of DIA/DIG but with focal areas of increased proliferative activity. DNA methylation profiling classified both tumors as an IHG (calibrated scores >0.9), and the RNA fusion panel detected CCDC88C::RAF1 and MTSS1::RAF1 fusions, respectively. No other variants were detected. **Conclusion:** We report two similar cases of infant glioma, each of which has mixed features of DIG/DIA and IHG. This suggests overlapping features between these subgroups

and therefore the need for further refinement of the infant glioma molecular classification. This also represents a significant clinical challenge in choosing the most appropriate therapeutic strategy. **Keywords:** Infant glioma – RAF1 fusion – infant-type hemispheric glioma – desmoplastic infantile astrocytoma/ganglioglioma

P120

TMEM119 immunoreactivity does not reveal any impact of microglia on radiological tumor growth or survival in patients with glioblastoma

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Introduction. Glioblastomas (GBMs) are highly aggressive tumors characterized by a heterogeneous microenvironment (TME), in which tumor associated microglia and macrophages (TAMs) are substantial components. These cells may play a central role in tumorigenesis and progression, making them potential targets for novel therapies. However, the specific functions of microglia in the context of GBM remain inadequately understood and warrant further investigation. **Objectives.** Our aim was to investigate the association between microglia, tumor growth, proliferation and overall survival [IN1] in human GBMs. **Materials & methods.** Radiological speed of growth was estimated for 91 GBM patients before treatment, dichotomized into faster- or slower-growing tumors. Standard immunohistochemistry was performed using TMEM119 (HPA051870, Sigma, US) to identify microglia, and the immunostaining was evaluated digitally using QuPath. Overall survival served as the primary endpoint for prognosis. **Results.** TMEM119 immunoreactive cells were observed in varying quantities across tumor samples, with a median ratio of 2.6%. Statistical analysis revealed no significant associations between TMEM119 immunoreactivity and tumor growth or overall survival. **Conclusion.** Our results suggest that the density of microglia, as assessed by TMEM119 immunoreactivity, does not significantly influence tumor growth or prognosis in patients with GBM. Further studies are necessary to elucidate the precise role of microglia in the pathophysiology of glioblastoma. **Keywords:** TMEM119 – microglia – glioblastoma – growth – survival

P121

Transcriptional profiling of cellular senescence in aged glioblastoma patients

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Introduction: Glioblastoma (GBM) is an aggressive brain tumor predominantly affecting older adults, with inevitable recurrence after standard treatment. The impact of aging-related factors, particularly cellular senescence, on tumor development and progression remains poorly understood due to underrepresentation of elderly patients in existing studies. **Objectives:** Here we aim to characterize

the senescence response in both tumor and microenvironment of elderly GBM patients (>70 years old) and establish a comprehensive molecular atlas of aged GBM. **Materials & methods:** We performed 10x Flex Fixed RNA-profiling on FFPE samples from GBM patients, low-grade glioma, and epilepsy controls. Senescent cells were identified using established gene lists and machine learning algorithms. Results were compared with published single cell and single nucleus RNA-sequencing data from younger GBM patients, neurotypical controls, and age-matched Alzheimer's disease cases. **Results:** Our dataset (snGlioAge) comprises 405,230 nuclei from 61 samples across 59 patients, including 49 GBM patients (ages 71-92), 5 low-grade glioma, and 5 epilepsy controls. Besides malignant and immune cells large clusters include neuronal cells. Analysis revealed high senescence scores in both malignant cells and microglia/macrophages, a finding validated in external datasets. Elevated microglia senescence was also observed across neurotypical controls and Alzheimer's disease cases, independent of chronological age. **Conclusion:** This comprehensive study establishes a reference dataset for aged GBM patients and demonstrates that senescence signatures are present in both tumor cells and the brain microenvironment, providing new insights into age-related factors in GBM. **Keywords:** Glioblastoma – senescence – microenvironment

P122

Two rare pediatric brain tumors diagnosed by methylation profiling

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Introduction: Pediatric brain tumors represent a heterogeneous and diverse group of rare neoplasms, often with ambiguous morphology and immunophenotype. We present two cases creating diagnostic difficulties resolved by methylation profiling. **Cases:** A 13-year-old female with a left occipital lobe tumor. The CT of the brain revealed a solid cystic lesion of 40mm with calcifications. Histopathological examination revealed a spindle cell and epithelioid tumor with high cellularity, high mitotic activity, and a rich network of hyalinized blood vessels and microcalcifications. IHC: GFAP(-), EMA(+), Olig2(+), CD56(-), CD34(-), Ki67 5-10%, BCOR(+/-), TLE 1+\-. The initial diagnosis was Synovial sarcoma. Methylation analysis classified the tumor as a CNS high-grade neuroepithelial tumor with MN1 alteration, MN1:BEND2 fusion subtype. In correlation with the histology, a diagnosis of Astroblastoma with MN1 alteration was made. A 17-year-old girl with frontal lobe tumor, suspected of echinoccocosis. CT demonstrated a solid-cystic 70mm tumor with intracerebral and meningeal components. Histopathologically, the tumor showed glial and neuronal differentiation, with focal necrosis, high mitotic index, and high cellularity, with leptomeningeal involvement. Moreover, polymorphous nuclei, clear cells, perivascular/stromal hyalinization, pseudorosettes, areas of oligodendroglioma-like differentiation, and scattered giant cells were seen. IHC: GFAP(+), Olig2(+), CD34(+/-), EMA (-), Ki67 20%, SMA(-), Synaptohysin(+), S100(+), NeuN&NFP (+/-), p53(-), IDH1R132(-), ATRX(+), BRAFV600 (-), H3G34(-). Our diagnosis was Anaplastic ganglioglioma. Heidelberg classifier showed the diagnosis of a Neuroepithelial tumor with Patz1 fusion. **Conclusions:** DNA methylation profiling was crucial for establishing both diagnoses. However, in the second case (an emerging entity), tumor grade, which defines a clinical approach, is based on morphology. **Keywords:** Neuroepithelial tumors – Patz1 – astroblastoma

P145

Distinct molecular profile and clinical outcome of oligodendroglioma, *IDH*-mutant, 1p/19q-codeleted and *TERT*p-wildtype: a grade 1 oligodendroglioma of young patients?

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Introduction: Oligodendrogliomas *IDH*-mutant and 1p/19q-codeleted often exhibit telomerase reverse transcriptase promoter (*TERT*p) mutations, which have been linked to telomere maintenance (TM) and tumor proliferation. Although there are a few reports on a *TERT*p-wildtype subset of these tumors in adolescents and young adults, the frequency, molecular characteristics and prognostic implications of *TERT*p-wildtype status in oligodendrogliomas remain elusive. **Objectives:** Since the spectrum of knowledge on *TERT*p-wildtype oligodendrogliomas is still extremely limited, we aimed at assessing the frequency, molecular characteristics, and clinical behavior of *TERT*p-wildtype oligodendrogliomas. **Materials & methods:** We retrospectively analyzed 166 *IDH*-mutant and 1p/19q-codeleted oligodendroglioma cases through comprehensive histopathological review and molecular analyses, including Sanger sequencing, DNA methylation profiling and whole-exome sequencing (WES). **Results:** A *TERT*p-wildtype status was determined in 20/166 cases (12.0%) and found associated with noticeably young age (*p<0.001*), WHO grade 2 (*p=0.003*), and the absence of additional DNA copy number variations (CNVs) beyond the entity-defining 1p/19q codeletion (*p<0.001*). Epigenetic profiling demonstrated *TERT*p-wildtype tumors forming a distinct subgroup at the utmost periphery of *TERT*p-mutant oligodendrogliomas. Methylation analysis of the upstream and proximal *TERT*p regions revealed that, in line with the absence of genetic alterations, epigenetic regulation does not favor *TERT* overexpression in *TERT*p-wildtype oligodendrogliomas. WES showed no TM-related genes alterations in *TERT*p-wildtype cases. Cox regression analysis confirmed *TERT*p-wildtype status as an independent prognostic factor for improved progression-free survival (PFS) (*p=0.009*). Conclusions: In conclusion, "oligodendroglioma, *IDH*-mutant, 1p/19q-codeleted and *TERT*p-wildtype" represents a distinct molecular subgroup associated with younger age and a more favorable clinical course compared to WHO grade 2 oligodendrogliomas. **Keywords:** Oligodendroglioma *IDH*-mutant and 1p/19q-codeleted – *TERT* promoter – methylation – progression-free survival – adolescents and young adults

P150

Nanopore sequencing for complete molecular work-up of CNS tumors

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Introduction: Since the publication of the 5th edition of the "WHO Classification of CNS Tumors" (2021), comprehensive molecular testing has become essential for the classification of many CNS tumors. Although this approach allows a more uniform tumor classification, performing several different molecular analyses such as NGS, methylome analysis, MLPA, pyrosequencing and FISH is associated with increased costs and often significantly prolongs routine diagnostics. **Objective:** We aimed to investigate whether nanopore sequencing can serve as an alternative method to replace all current standard molecular analysis methods. **Patients and methods:** Fresh or frozen tissue from 78 CNS tumors - 58 retrospective and 20 prospective cases - was analyzed on a p24 device using PromethION flow cells from Oxford Nanopore Technologies. WGS results were generated within 72 hours and analyzed for pathological sequence alterations according to the Clinvar and COSMIC databases. Methylation-based tumor classification was performed using the locally run classifier

CrossNN and the online platform MNP-Flex. The results were compared with the findings of routine diagnostics and the final diagnosis. **Results:** In 75 CNS tumor samples, tumor-cell content was sufficient for further investigation. The methylationbased tumor classification matched the final diagnosis in 69 (MNP-Flex) and 67 (CrossNN) cases. In all methylation-based inconclusive samples, the WGS results showed the diagnostically relevant molecular changes that were sufficient for the final diagnosis. **Conclusion:** PromethION-based WGS is an attractive analysis that reveals all diagnostically relevant DNA-alterations within five working days and should therefore be considered a new standard molecular analysis method for CNS tumor diagnostics. **Keywords:** nanopore – methylation - whole-genome - CNS - tumor

P159

Carbonic anhydrase 12 as a possible target structure for intracavitary radioimmunotherapy in glioblastoma treatment: An immunohistochemical evaluation

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Introduction: Patients diagnosed with glioblastoma still face a very poor prognosis. Intracavitary radioimmunotherapy (iRIT) has recently shown promising results as a therapy option to the current standard of care. iRIT, however, is dependent on glioma selective target structures. One possible target structure is the cell surface protein Carbonic Anhydrase 12 (CA12), which is expressed on gliomas yet not on healthy brain cells. **Objective:** Aim of this study was to examine the expression of CA12 in glioblastomas and evaluate the potential of CA12 as a possible target structure. We also correlated our findings with the individual MGMT methylation and the EPIC-methylation profile subtype. **Materials & methods:** We selected 105 glioblastoma samples (IDH-wt, WHO Grade 4) from the archive of the Neuropathology Department at TUM between 2020-2024. The FFPE-specimens were immunohistochemically stained with a monoclonal mouse antibody targeting CA12 (OriGene, clone UMAB121). Objectives were CA12 expression (yes/no), percentage of stained area and staining intensity. Potentially noticeable staining patterns were recorded. **Results:** 93,3% of glioblastoma samples were positive for CA12. Within this population, we observed an average tumor cell positivity of 35,99% per sample. MGMT methylation as well as mesenchymal subtype seemed to be lightly associated with CA12 positivity. We observed a gathering of CA12 positive cells next to necrotic areas. **Conclusion:** Our results prove CA12 to be a favorable target for iRIT. A potential association between predictors of prolonged survival, namely MGMT methylation and mesenchymal subtype, and CA12 expression merits further exploration. The results offer encouraging support for continued investigation into the therapeutic potential of CA12 expression in clinical studies. **Keywords:** CA12 – intracavitary immunotherapy – glioblastoma – neuro-oncology

15. Tumors - others

P123

A rare ZNF532::NUTM1 fusion-driven pediatric brain tumor

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Introduction & background: NUT-fusion tumors represent a rare, aggressive type of poorly differentiated carcinomas, characterized by chromosomal rearrangements involving the nuclear protein in testis gene (NUTM1), commonly located in midline structures of the head, neck and thorax, with several known fusions. **Clinical case:** A 3-year-old boy presented with increasing restlessness and vomiting 6 months before the diagnosis of an embryonal tumor NEC in the left lateral ventricle measuring 5 x 3 cm with supra-/infratentorial and spinal leptomeningeal enhancement. Histological examination showed a highly cellular tumor composed of small uniform cells with round nuclei, distinct nucleoli and a scant cytoplasm. The cells were arranged in a sheet-like pattern without a discernible background. Immunohistochemically, expression of Olig2, Chromogranin A, NCAM, BCOR and EGFR was present, synaptophysin and SOX10 were positive in a fraction of cells. DNA methylation profiling using the DKFZ and the Bethesda CNS tumor classifier did not match with any methylation class. Archer FUSIONPlex panel showed a ZNF532::NUTM1 fusion. After initial total resection chemotherapy was started based on the HIT-MED-Guidance protocol until it was switched to the Scandinavian sarcoma protocol due to a mixed response. Treatment was accompanied by intraventricular therapy. Multifocal metastatic progression occurred prior to planned craniospinal irradiation (CSI). 6 months after CSI, during maintenance intraventricular therapy, MRI showed no evidence of tumor progression or new lesions. **Conclusion:** Rare CNS embryonal tumors NEC should be screened for NUT fusions. Currently no standard therapy is available. In this case, the tumor shows good response to radiotherapy. **Keywords:** Pediatric neurooncology – precision medicine – embryonal tumor – NUTM1 fusion

P124

Characterization of the immune microenvironment in atypical meningiomas

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Background: Meningiomas are the most frequent primary intracranial tumors. The high incidence of grade 2 meningiomas (or atypical meningiomas) and their tendency to recur make them a challenge for patients and healthcare systems. **Objective:** To evaluate if a better knowledge of the immune microenvironment of meningioma may help in planning treatments, we carried out a study on the tumor immune infiltrate looking for a role as a prognostic/predictive marker. **Materials & methods:** We evaluated the presence of different classes of immune infiltrates through single and double immunohistochemical and immunofluorescence analysis in 77 atypical meningiomas. Finally, we evaluated the focal loss of H3K27-me3, a recognized marker of aggressiveness in meningiomas. Specialized software for automated analysis enabled us to validate the obtained data. Results: We find out a significant presence of T lymphocyte infiltrates widespread in the tumor, mainly CD8+, while B lymphocytes are mainly localized at the interface with healthy nervous tissue. Macrophages were subdivided into two populations: M1 (anti-tumor) and pro-tumoral M2, more prevalent. CD11b+ monocytes are mostly expressed in blood vessels, while suppressive myeloid immune cells CD14+ are widespread in the tumor. In contrast to what was seen for the other infiltrates, the presence of few monocyte infiltrates (CD11b+ e CD14+) leads to a reduction in both survival and the time to onset of relapse. **Conclusions:** Atypical meningiomas presents a highly infiltrated tumor microenvironment and the study of this can provide valuable insights into the prognostic and predictive profile of disease progression. **Keywords:** Meningioma – immune profile – tumor microenvironment – immunohistochemistry

P125

Characterization of the immune profile in a cohort of brain metastases

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Introduction: Brain metastases (BMs) are the most common intracranial tumors, affecting about 20% of advanced cancer patients. Currently, immune checkpoint inhibitors (ICTs), targeting PD-L1/PD1 and CTLA-4 axes, shows most favorable outcomes for patients. The response is even improved when tumors exhibit mismatch-repair system (MMR) deficiency. Furthermore, the tumor microenvironment is characterized by the presence of infiltrating lymphocytes and myeloid cells, and this could influence tumor progression and its response to therapies. **Objectives:** This study aims to evaluate ICs (PD-1/PD-L1, CTLA-4 and CD28) and MMR proteins (MSH6, MSH2, MLH1 and PMS2) and to characterize the lymphocytes, macrophages, myeloid precursors, and NK cells in BMs. **Materials & methods:** Immunohistochemical analysis was performed on 75 BMs from lung, ovarian and colorectal cancer, operated at our institute from 2018 to 2020. **Results:** Tumor-infiltrating lymphocytes are concentrated in tumor stroma and around vessels, while tumor-associated macrophages and myeloid precursors (CD14+, CD11b+) are mostly present at the interface between brain parenchyma and tumor mass. NK marker CD16 showed positive cells mostly inside vessels in both tumor and normal brain tissue. Loss

of MMR proteins expression was assessed in almost 25% of the cases, probably related to the overexpression of the PD1/PDL1 axis. CTLA-4 expression was found in 10% of the samples, while CD28 in 50% of cases. **Conclusions:** Our data confirm that secondary brain tumors trigger an immune reaction modifying the microenvironment. Understanding immune cells behavior and localization, may help to predict tumor progression and guide personalized therapeutic strategies for patients. **Keywords:** Brain metastases – immunotherapy – tumor microenvironment – immune checkpoints – immunohistochemistry

P126

Embryonal tumor with multilayered rosettes: A case report of a rare and aggressive pediatric brain tumor

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Introduction: Embryonal tumors with multilayered rosettes (ETMR) are rare, highly aggressive pediatric central nervous system tumors, primarily affecting infants and young children. They are characterized by small round blue cells, multilayered rosettes, and amplification of the C19MC region on chromosome 19q13.42. Due to their rarity and poor prognosis, further documentation of ETMR cases is essential to improve understanding and management. **Case presentation:** We present the case of a 22-month-old female infant who developed vomiting, somnolence, and generalized hypotonia over three days. Brain imaging revealed a large supratentorial tumor with mass effect and midline shift. She underwent gross total resection, and histopathological analysis showed a biphasic architecture with hypercellular areas of small blue cells, perivascular arrangements, and rosette formation, along with neuropil-like fibrillary regions and large necrotic areas. Immunohistochemistry was positive for synaptophysin and NeuN in the neuropil-like matrix, with focal GFAP positivity and retained INI1 expression. DNA methylation profiling confirmed the diagnosis of ETMR, C19MC-altered. **Discussion:** ETMR is often misdiagnosed as other embryonal or high-grade gliomas due to overlapping histological features. Molecular profiling is essential for accurate classification, as in this case. Despite aggressive surgical resection, the patient experienced rapid neurological decline, early recurrence, and tumor progression, highlighting the limited effectiveness of current treatment strategies. **Conclusion:** This case underscores the aggressive nature, diagnostic challenges, and poor prognosis of ETMR. Given the high recurrence rate and lack of effective therapies, further research into molecularly targeted treatments is crucial to improve outcomes for affected children. **Keywords:** Embryonal tumor with multilayered rosettes, C19MC amplification – DNA-methylation analysis

P127

Expanding the clinicopathological spectrum of glioneuronal tumors with NTRK gene rearrangement - report of three cases

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Background: Glioneuronal tumors harboring receptor tyrosine kinase (RTK) gene fusions have been recently described in the literature, however, data regarding their pathological presentation and clinical behavior are limited. Hereby, we report three cases of NTRK-fused glioneuronal tumors. **Case reports:** A 29-year-old woman (Case 1) and an 8-year-old girl (Case 2) presented with frontal lobe tumors, while Case 3 (5-yeard-old boy) had a month history of a posterior fossa lesion radiologically suspicious of dermoid cyst. Histologically, Case 1 showed a low-grade glioneuronal tumor featuring by oligodendroglia-like cells and many ring-like nuclear clusters. In contrast, Case 2 and Case 3 had rather similar patterns and consisted predominantly of primitive-looking Olig2-positive cells with areas of neurocytic and ganglionic differentiation, raising the possibility of CNS neuroblastoma, *FOXR2*-activated. DNA Methylation array (Brain classifier v12.8, DKFZ) profiled both supratentorial tumors as "MC Glioneuronal Tumor, Subtype A", while the posterior fossa tumor showed no match for existing classes. Next generation sequencing detected RTK fusions in all three instances, including an in-frame *TPM3::NTRK1* inversion, an *AGAP1::NTRK2* fusion and *TPR::NTRK1* fusion, respectively. On follow-up, Case 1 is tumor-free 22 months after the surgery. Case 2 and 3 are stable on MRI (8 and 5 months) following gross total resections with adjuvant radiotherapy and maintenance chemotherapy as per SIOP-PNET5 protocol. **Conclusion:** NTRK-fused glioneuronal tumors encompass a clinically and morphologically heterogeneous tumor group, including infratentorial cases with embryonal-type morphology. Decision about the therapeutic protocol may be difficult in such unclassifiable cases. NTRK-inhibitors may offer a therapeutic opportunity for the future. **Keywords:** Pediatric CNS tumor – NTRK gene rearrangement – MC glioneuronal tumor subtype A – neuroblastoma – oligodendroglia-like cells – DNA methylation profiling

P128

Expanding the spectrum of central nervous system neuroepithelial tumor with PLAGL1 fusion

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Background: Neuroepithelial tumors with PLAGL1 fusion (NET-PLAGL1) have recently emerged as a distinct category of CNS tumors with ependymal differentiation. **Objectives:** We report clinical,

neuropathological, and molecular characteristics of three cases. **Methods:** The cases concerned three 22-, 29-, and 24-month-old boys with large frontal tumors. Case #1 and #2 were originally diagnosed as supratentorial ependymoma grade 2 and 3, respectively. In the third recent case the integrated diagnosis of NET-PLAGL1 was given. All were analyzed with methylation profiling, and RNA sequencing. **Results** and #2 displayed ependymoma-like morphological and immunophenotypical features, with interspersed ganglion-like cells in case #2. Case #3 had an unusual appearance with neuropil-rich islands containing neurocytic cells. All cases were classified as NET-PLAGL1 (DKFZ Classifier version v12.5, score 0.99). In case #1 and #2, RNAseq identified a *EWSR1::PLAGL1* fusion, where *PLAGL1* exon 5 was rearranged with *EWSR1* exon 8 and exon 6 respectively. Case #1 exhibited a 62 Mb deletion on chromosome 6q encompassing *PLAGL1* locus by chromosomal microarray. The third case harbored a novel *MN1(ex1)::PLAG1(ex5)* fusion. All the chimeric proteins were predicted to retain the functional zinc-finger domain of PLAGL1 or PLAG1. Patient #1 received radiotherapy and chemotherapy, while patient #2 underwent only radiotherapy; both remain disease-free after 52 and 74 months. Case #3, after gross resection and radiotherapy, is doing well 12 months post-diagnosis. **Conclusion:** This study expands the pathological and molecular spectrum of NET-PLAGL1 tumors, with the unprecedented findings of neurocytic differentiation and a rearrangement involving *MN1* and *PLAG1* genes. **Keywords:** Neuroepithelial tumors with PLAGL1 fusion – *MN1::PLAG1* fusion, supratentorial

P129

Implications of cIMPACT-NOW update 8 on previously classified meningiomas: A comparative study

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Introduction: Recent molecular advances have improved our understanding of the biological behavior of meningiomas; however, reliable prognostic systems are still under development. **Objectives:** This study aims to assess the implications of the recently introduced cIMPACT-NOW update 8 (cINU8) compared to the Integrated Molecular-Morphologic Score (IMMS) system and 2021 WHO grading. **Methods:** A large departmental cohort of WHO grade 1-3 meningiomas with available DNA Methylation array data (DKFZ, Brain Tumor Classifier) were stratified by IMMS and cINU8, and the changes were studied. **Results:** The study included 219 meningioma cases (mean age: 56 years). Of the 126 histologically grade 1 meningiomas, 103 (81.75%) remained low risk after molecular investigations. Twenty-three (18.25%) cases were upgraded to WHO grade 2 after the application of cINU8, of which 13 (10.3%) were low risk by IMMS. Fourteen (11.11%) histologically benign cases with a subsequently detected higher-risk molecular profile could have been missed if the cINU8 testing criteria were strictly followed. Of the 87 histologically grade 2 meningiomas, 27 (31%) showed no indication of high-risk copy number variation, while 48 (55.2%) were classified as intermediate risk and 12 (13.8%) as high risk by IMMS. Four out of six grade 3 tumors fell into the high-risk IMMS category. **Conclusion:** The application of cINU8 led to a change in grade in 18.25% of the WHO Grade 1 cases. Approximately one-third of the histological grade 2 cases did not show a high-grade molecular profile. Additionally, the study highlights a gap in the cINU8 molecular testing criteria, suggesting the need for refinement to address testing scenarios. **Keywords**: Meningioma – cIMPACT-NOW – integrated molecular-morphologic score – WHO classification – molecular neuropathology – grading

P130

Incidence of brain metastases in eastern Finland between 2003-2024

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Introduction: Brain metastases (BM) are the most common central nervous system tumors. Their incidence is expected to rise due to aging populations and improved cancer survival. Data on BM vary, and multiple specialties are involved in patient care. Objectives: to assess the incidence and characteristics of MRI-confirmed and histopathologically verified BM in the Eastern Finland collaborative region. **Materials & methods:** Patients with MRI-confirmed BM (February 2003–January 2024) were identified from the local PACS database, as MRI is standard in BM diagnosis. Histopathological records confirmed BM, and the Finnish Cancer Registry (FCR) provided population-based cancer incidence data. **Results:** We identified 689 patients with MRI-positive BM, with histopathological confirmation in 623 cases. Malignancy in the remaining 66 was verified through prior medical records, imaging, or laboratory analyses. BM originated from lung cancer (30%), breast cancer (24%), melanoma (10%), colorectal cancer (6%), and renal cell carcinoma (6%). Between 2004 and 2022, FCR recorded 80,532 primary cancer cases in the region, with 618 MRI-confirmed BM cases, corresponding to an incidence of 0.77%. **Conclusion:** BM significantly impacts cancer patients' morbidity and mortality. As the Eastern Finland collaborative region represents ~15% of Finland's population, these findings offer generalizable insights into BM incidence. **Keywords:** Brain metastases – brain imaging – intracranial metastatic disease – cancer – incidence

P132

Meningeal foamy cell tumor. Differentiating between histiocytosis, xanthomatous meningioma and tumefactive m. Whipple.

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Background: Foamy cell tumors in the central nervous system are rare. Within the differential diagnosis when located in the meninges there are three main differential diagnoses that have to be worked up as they are followed by specific therapy: histiocytosis, xanthomatous meningioma and tumefactive M. Whipple. **Clinical:** A 46-year-old Caucasian woman had several epileptic seizures with a focal origin in a two-week period. These seizures were treated effectively with levetiracetam. Neurologic investigation at the time of neurosurgical presentation showed no deficits. A cerebral MRI showed an extra-axial left frontal tumor consistent with a falx meningioma although there was only a

patchy enhancement with gadolinium. Histopathology showed a predominant foamy cell proliferation intermingled by several meningothelial cells and some chronic inflammation. The foamy cells expressed several markers of histiocytic origin. Molecular analysis with NGS and Fusion-gene analysis failed to demonstrate specific mutations or fusion genes favoring a histiocytosis. Methylation profiling gave no hint for diagnosis of specific histiocytosis. Instead, a relatively low score for the diagnosis meningioma was found (Heidelberg and Bethesda classifier). PCR analysis for M. Whipple was negative. In histological staining's no microorganisms were found. **Conclusion:** Definite diagnosis was not possible although histiocytosis and tumefactive M. Whipple were ruled out. Best fitting diagnosis would be xanthomatous meningioma. **Keywords:** Histiocytosis – xanthomatous meningioma – M. Whipple

P133

Meningioangiomatosis-associated sarcomas: insights on the molecular mechanisms

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Background: Meningioangiomatosis (MA), a poorly characterized cortical lesion comprising small vessels surrounded by a spindle cell proliferation, may occasionally be associated with spindle cell sarcomas. **Objectives:** We sought to gain insights into the molecular mechanisms underlying these rare lesions. **Methods:** Tissue specimens from three patients with MA-associated sarcomas (MA-S) (age 4, 6 and 27y) and seven patients with pure MA (median age 11y, 3-14y) were analyzed by NGS panels, and RNAsequencing. None of the 10 patients had signs of NF2. **Results:** MA-S, beside the MA component, showed extensive areas of CD34-positive spindle cells arranged in a fascicular and storiform pattern, with focal nuclear pleomorphism and brisk mitoses. Both pediatric cases harbored NRAS p.(Q61K) mutation, combined to NF2 p.(R196*) mutation in one case and TP53 p.(P152T) mutation in the other. The young adult MA-S carried a novel SPECC1L::RAF1 fusion and a PBRM1 p.(R522*) mutation. The above-described alterations were documented in both the sarcomatous and MA components in each case. SMARCB1-deficient undifferentiated areas were additionally present in one pediatric and in the young-adult MA-S and were associated with a novel SMARCB1::CABIN1 fusion and a homozygous deletion of 22q11.23 (SMARCB1), respectively. All pure MA cases showed typical histological features and carried variants in NRAS p.(Q61K) (n=2), NF2 (c.1341-2A>G) and NF1 p(R2517*) (1 case each), as well as few CNA (n=4), including deletions at 22q (n=2). **Conclusion:** The identification of NRAS p.(Q61K) mutation in a subset of pure MA and in 2/3 MA-S is novel and suggests

a possible evolution from MA to rare spindle cell sarcomas. **Keywords:** Meningioangiomatosis – spindle cell sarcomas – meningioangiomatosis-associated sarcomas – NRAS p.(Q61K) – RAF1 fusion

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Neurofibromatosis Type 2 mutations are not predicted by merlin immunoreactivity

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Introduction: The latest cIMPACT-NOW update recommends that meningiomas with 1p deletions and concurrent neurofibromatosis type 2 (NF2) mutations and/or 22q monosomy should be graded as minimum World Health Organization (WHO) grade 2 tumors. Alterations in NF2 are reported in more than half of sporadic meningiomas, underlining the importance of this gene in meningioma tumorigenesis. NF2 encodes the tumor suppressor protein merlin, consisting of three domains: N-terminal, C-terminal and central helix. Hence, one could assume NF2-mutated meningiomas to express less merlin, although previous immunohistochemical studies have described various merlin immunoreactivity. **Objectives:** We hypothesized that merlin could be used as a surrogate marker for NF2 mutations. Accordingly, our aim was to relate merlin immunoreactivity to NF2 status. **Materials & methods:** Next Generation Sequencing was performed on tumor specimens from twenty patients with WHO grade 2 meningiomas to detect mutations in the NF2 gene. The specimens underwent immunohistochemical analyses with antibodies directed against the N-terminal and C-terminal of merlin. Immunoreactivity was assessed by a neuropathologist. Mann Whitney U test was applied to investigate any relations between NF2 mutation and merlin immunoreactivity. **Results:** NF2 mutations were detected in 55% of the tumor specimens. All specimens had a moderate to strong merlin immunoreactivity, regardless of antibody. There were no significant relations between merlin immunoreactivity and NF2 mutation ($p > 0.6$). **Conclusion:** Merlin immunoreactivity is not an appropriate surrogate marker for NF2 mutations, as merlin was abundantly expressed in all included specimens regardless of applied antibody and there were no significant relations to NF2 status. **Keywords:** Meningioma – merlin – NF2 – immunohistochemistry – cIMPACT-NOW

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PLAGL amplified neuroepithelial tumor: an interesting case report with long clinical follow-up and sarcomatous transformation

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Introduction: PLAGL amplified neuroepithelial tumors (NET-PLAGL) represent a newly identified methylation class characterized by distinct ependymal differentiation. Their histomorphological,

clinical, radiological, and prognostic features have not yet been fully elucidated, and the diagnosis without methylation analysis is challenging. We report a rare case with sarcomatous transformation and a 26-year follow-up. **Clinical:** The patient presented at the age of 12 with hearing and vision loss, leading to the detection of a 7.5cm mass in the parieto-occipital region. Biopsy revealed relatively monotonous neoplasm leading to a diagnosis of PNET. The patient underwent cisplatin-etoposide chemotherapy and craniospinal radiotherapy, achieving remission. Twenty years later, the patient developed visual field loss and tinnitus. Imaging revealed a 4.3cm contrast-enhancing lesion in the resection cavity. Biopsy showed a tumor with perivascular pseudo rosettes, low mitotic activity, GFAP and dot-like EMA positivity consistent with grade 2 ependymoma. No further treatment was administered for the totally resected lesion. Three years later, MRI showed an 8mm contrast-enhancing lesion with spinal seedings. Findings were consistent with prior recurrence. The patient received radiotherapy and chemotherapy. Fifteen months later, a lateral ventricle mass was resected, showing similar ependymal differentiation and sarcomatous transformation with mitoses and necrosis. Methylation analysis identified the tumor as a NET-PLAGL, and EWSR translocation was shown by FISH. **Conclusion:** NET-PLAGL is characterized by pronounced ependymal differentiation and distinct methylation profile. However, sarcomatous transformation has not been previously described. Gross total resection is key to prognosis, and our case with a 26-year-long clinical follow-up provides valuable insights into this rare tumor. **Keywords:** PLAGL amplified neuroepithelial tumors – methylation analysis – sarcomatous transformation – ependymal tumors

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Re-evaluation of meningioma grading using molecular parameters

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Introduction: The molecular diagnostic criteria for meningiomas in the fifth edition of the WHO Classification of CNS tumors (WHO CNS5) were ambiguous in the application. The Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy-Not Official WHO (cIMPACT-NOW) published a recommendation for a novel scheme for meningioma grading (Sahm F. Brain Pathology 2024). **Objectives:** Reevaluation of the grading of previously diagnosed grade 2 and 3 meningiomas. **Materials & methods:** We selected 35 consecutive surgical cases of those tumors from 2021 to 2024. Using formalin-fixed paraffin-embedded tissue, we investigated the extent of chromosome 1p loss in these meningioma cases with multiple ligation-dependent probe amplification (MLPA) analyses. **Results:** 1p loss was only detected in 7/19 (37%) tumors of grade 2 meningiomas assessed by the histological criteria of WHO CNS5, suggesting that 63% of grade 2 meningiomas could be downregulated by a novel molecular marker of 1p loss. Furthermore, 11/13 (85%) tumors of grade 3 meningiomas showed 1p loss. MLAP analyses detected *CDKN2A* HD (homozygous deletion) in 6/13 (46%) tumors of grade 3 meningiomas, most of the HD concurrent with 1p loss (5/6 tumors: 83%). Conversely, Sanger sequencing detected *TERT* promoter mutations only in 2/13 (15%) tumors of grade 3 meningiomas. **Conclusion:** The findings suggested that current WHO CNS 5 criteria may over-diagnose grade 2 meningiomas and that 1p loss could be a broader molecular marker for dividing meningiomas into lower-risk and higher-risk groups. Further clarification is required for a better grading system for meningiomas. **Keywords**: WHO – meningioma – grading – 1p loss

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Ruptured intracranial epidermoid cyst: An unexpected discovery

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Introduction: Intracranial epidermoid cysts are rare but benign tumors. Although benign, they are often adherent to neurovascular tissues, making their surgical removal particularly complex. More than a third of these tumors are located at the cerebellopontine angle. **Clinical case:** The brain of a 37-year-old man was sent for neuropathological analysis following a forensic autopsy to the Department of Pathology at Cliniques universitaires Saint-Luc. Among the few clinical details provided, it was noted that the man reportedly died following a neurosurgical intervention, with no further information. During the autopsy, a site of occipital craniectomy was identified, below the cruciform eminence. A subdural hematoma of the left middle and posterior cranial fossa was also observed. Neuropathological analysis of the cerebellum sections revealed a cystic lesion with a maximum diameter of 4 cm, hemorrhagic in its center and surrounded by a whitish capsule. The microscopic examination confirmed the cystic lesion as a ruptured epidermoid cyst. Despite our requests, no additional information regarding the neurosurgical intervention was provided, making it impossible to interpret the timing of the cyst rupture. **Conclusion:** This case illustrates a rare and benign tumor that can have potentially fatal consequences. It also highlights the importance of good communication between the various medical professionals involved to ensure accurate interpretation. **Keywords:** Epidermoid cyst – intracranial tumor – ruptured cyst – neuropathology – neurosurgery

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The conundrum of CNS PATZ1-fused tumors: A unique DNA-methylation signature for a variety of clinico-radio-pathological aspects

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Introduction: Recently, a NET with PATZ1 fusions (NET-PATZ1) has been described, but has not been yet added in the new WHO classification. PATZ1 may be fused to two different genes (EWSR1 and

MN1). In soft tissue, EWSR1::PATZ1 fusions have been identified in a subset of small round cell sarcomas. In the CNS, one hundred of cases have been published in the literature but no specific clinicopathological profile has been drawn with a wide variety of morphologies. Genetic analyses have evidenced a specific overexpression of GATA2 gene in this tumor type. **Objectives:** To better characterize NET-PATZ1, we performed a comprehensive retrospective analysis of a series of patients. We also analyzed the sensitivity and specificity of GATA2 immunohistochemistry in a control cohort of its differential diagnoses. **Materials & methods:** We characterized twelve cases with a PATZ1 fusion including with clinical, radiological, histopathological ultrastructural, immunohistochemical, genetic and epigenetic analyses. **Results:** There were no pathognomic clinical (pediatric and adult cases), radiological (hemispheric, periventricular and pineal locations, with a majority of solid and cystic lesions) and histopathological (polymorphous tumors with a variety of patterns: ependymoma-, glioneuronal and sarcoma-like features) pattern. Ultrastructural analyses evidenced glioneuronal features. GATA2 expression was observed in 6/9 tested tumors, without any immunopositivity for other glioneuronal or mesenchymal tumors. Morphological/genetic correlation showed a tendency that tumors harboring EWSR1 fusions presented sarcoma-like features. **Conclusion:** Our work highlights that NET-PATZ1 constitute actually a diagnostic challenge for neuropathologists. Our study brought additional findings arguing for a pluripotent origin cell. **Keywords:** PATZ1 – glioneuronal – origin – radiological – ultrastructural

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Third ventricle choroid plexus tumor with ependymal differentiation: Two cases and literature review

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Introduction: Herein, we report two cases of choroid plexus tumor with ependymal differentiation. **Clinical case:** First case: An infant girl (three-month-old) diagnosed with macrocephaly from birth was found to have a neoplasm in the third ventricle, necessitating surgical intervention. In this case, there is a notable papillary proliferation with increased epithelial stratification on the papillae surfaces. Solid area, increased cellular density and nuclear pleomorphism are focally observed. Some solid growth areas show ependymal differentiation forming true rosettes in a neuropilic background. Although there is no significant necrosis or brain invasion, mitotic activity is increased (10/10 HPF). Immunohistochemically, GFAP shows ependymal differentiation without staining in papillary structures; OTX2 and Transthyretin are positive in papillary structures but negative for ependymal differentiation. The morphological and immunohistochemical findings are consistent with choroid plexus carcinoma with ependymal differentiation (WHO CNS5 grade 3). Second case: A one-month-old infant girl was found to have a contrast-enhancing mass in the third ventricle on imaging, which led to surgery. There is a tumor characterized by papillary configuration and choroid plexus epithelial proliferation showing mild to moderate atypia, without necrosis, brain invasion or solid areas. It is noted that some areas of the choroid plexus epithelium showed ependymal differentiation. GFAP shows ependymal differentiation. Ki67 proliferation index is 30%. The morphological and immunohistochemical findings are consistent with atypical choroid plexus papilloma with ependymal differentiation (WHO CNS5 grade 2). **Conclusion:** To date, few choroid plexus papillomas with ependymal differentiation have been identified, while atypical papilloma and carcinoma with ependymal differentiation remain undescribed. Due to their rarity, we anticipate these two cases will

significantly contribute to the literature. **Keywords:** Ependymal differentiation – choroid plexus papilloma – third ventricle

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Novel analytical technologies for precision medicine of cranial-spinal sarcomas: A pilot study on mesenchymal chondrosarcoma (MCS)

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Introduction: Cranial-spinal sarcomas are rare cancers mainly affecting children and young adults with poor outcomes. They arise both from bone and soft tissue origin in the spine, nerves and cranium. Their poor survival rate is due to several factors including: 1) A high misdiagnosis rate and 2) Lack of targeted treatments due to a poor molecular understanding of tumor behavior. Over 25% of sarcomas are defined by fusion genes, which are often the driving molecular alteration, and thus an attractive target for diagnostic and therapeutic interventions. **Objectives**: This project focuses on improving the diagnosis and understanding of fusion-driven cancers through novel analytical technologies including a fusion-specific RNA in situ hybridization (ISH) probe and long-read whole genome sequencing (WGS). We are piloting the above approaches on an aggressive sarcoma with no targeted treatments, mesenchymal chondrosarcoma (MCS) characterized by the HEY1-NCOA2 fusion gene. **Materials & methods**: A BaseScope ISH probe was developed targeting the fusion break point. Validation was performed on a fusion inducible cell line and in human tissue. Five fusion-confirmed MCS cases were sequenced using Nanopore long-read WGS. **Results:** BaseScope ISH probe can spatially resolve HEY1-NCOA2 in a cell line and in human tissue. Long-read WGS identifies diverse fusion gene break points and additional co-mutational signatures, providing a useful tool to characterize fusion gene structure and behavior. **Conclusion:** Novel analytical technologies, including BaseScope ISH probe and long-read WGS provide valuable tools to characterize fusion gene function and could be used to aid in precision medicine approaches for rare sarcomas. **Keywords:** Cranial-spinal sarcoma – long-read sequencing – Nanopore – BaseScope – in situ hybridization – precision medicine

P141/SY13

Whole genome sequencing enhances the diagnostic yield in central nervous system tumors: The KCH neuropathology department experience

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Background: Accurate diagnosis and prognostic stratification of central nervous system tumors remain a challenge due to their molecular complexity, which often requires parallel running of diverse testing methods. **Objectives:** We aimed to evaluate the added diagnostic value of whole genome sequencing (WGS) compared to standard-of-care molecular tests. **Methods:** Fresh brain tumor samples from 255 patients (102 pediatric/TYA, 153 adult) were analyzed by multimodal NGS panel (covering 305 DNA and 76 RNA targets) and DNA methylation array (DKFZ). Comparative analysis with WGS data was

performed including detection of small nucleotide (SNVs) and structural variants, loss of heterozygosity and germline findings. **Results:** Multimodal panel detected diagnostic variants in 86.7% of the cases, with a total of 453 pathogenic SNVs and 58 gene fusions. WGS confirmed 96.7% of the known pathogenic SNVs and found an additional 90 previously undiscovered variants. All diagnostic fusions were confirmed by WGS. Twenty-four cases had no driver SNVs or gene fusions but were confidently profiled by methylation array. Germline variants were detected in 26 cases (identification rate of 10%). Four cases had high tumor mutational burden (13-189 mut/Mb). Methylation array appeared sensitive for copy number variation assessment, apart from chromoanagenesis events (33 cases) and genome-wide ploidy gains (40 cases). **Conclusion:** WGS offers a single-test approach with improved diagnostic utility over the standard-of-care tests. The added value was most apparent in detection of previously unknown germline variants and cases with high TMB that also altered patient management. WGS also returned superior structural variant information compared to methylation array. **Keywords**: Whole genome sequencing – diagnostic utility – gene fusion – germline variant – copy number variation – tumor mutational burden

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Novel approach for rapid immunohistochemistry of tumors in the central nervous system

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Introduction: Rapid immunohistochemistry can accelerate intraoperative diagnostics and support timely clinical decision-making. Definitive diagnostics beyond hematoxylin-eosin staining require overnight processing, which means that accurate conclusions are not yet possible during surgery. This mainly concerns lymphomas and the exact determination of malignancy. **Objective:** This study aimed to evaluate a novel approach for staining intraoperative tissue samples that is faster and more informative, to determine its potential for integration into rapid routine staining procedures. **Materials & methods:** We collected 129 intraoperative tissue samples. Sections were prepared, frozen, fixed with Delaunay fixing solution and stained with various polymerized horseradish peroxidase-conjugated antibodies such as Pan-CK, GFAP and CD20 according to our protocol. Staining intensity was assessed on a scale from 0 to 3+: 0 indicated absent or non-specific staining, 3+ represented strong, specific staining with minimal background. **Results:** GFAP demonstrated reliable signals in 69.2% of cases graded as 3+, 23% achieved 2+, while only 3.8% were classified as 1+ or 0. Pan-CK proved similarly robust, with 76.0% of samples graded 3+, 8% assessed as 2+, 16% classified as 1+ or 0. CD20 showed greater variability: 42.9% of cases were graded 3+, 7.1% were assessed as 2+, and 28.6% graded 1+. **Conclusion:** These results highlight the potential of rapid immunohistochemistry to improve diagnostic workflows. In the future, additional antibodies should be evaluated to enable more comprehensive differentiation between tumor entities and improve patient health. With such advances, rapid and accurate intraoperative diagnosis could become possible within minutes, enabling immediate, customized treatment for patients after surgery and reducing unnecessary interventions. **Keywords:** Rapid immunohistochemistry – Pan-CK – CD20 – GFAP –neuro-oncology – central nervous system